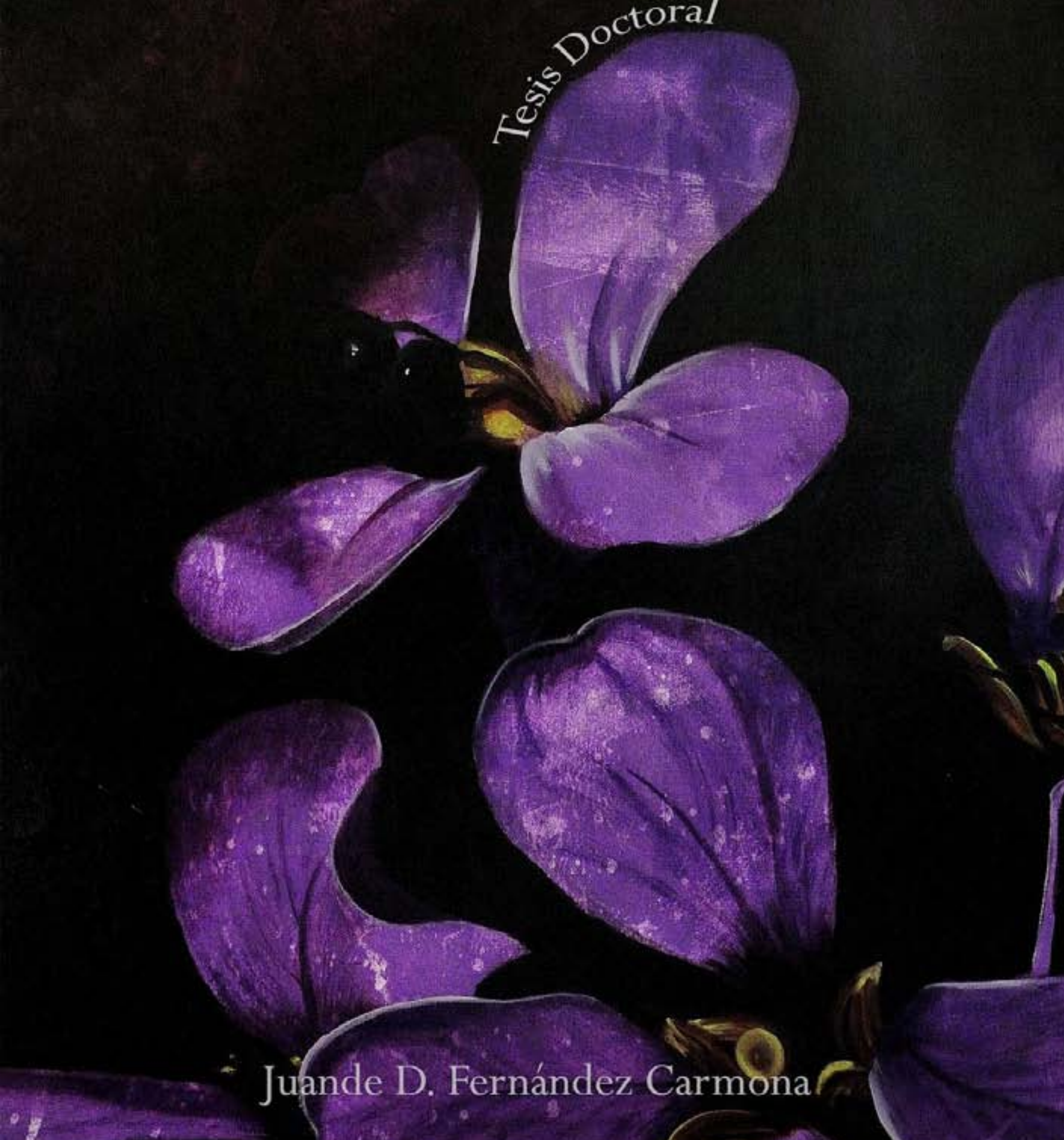
Variación espacial en la polinización y el éxito reproductivo de una herbácea endémica: implicaciones para su conservación

Tesis Doctoral



Juande D. Fernández Carmona

VARIACIÓN ESPACIAL EN LA POLINIZACIÓN Y EL ÉXITO REPRODUCTIVO DE UNA HERBÁCEA ENDÉMICA: IMPLICACIONES PARA SU CONSERVACIÓN

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Centre de Recerca Ecològica i Aplicacions Forestals (CREAF)
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Memoria que el Licenciado Juan de Dios Fernández Carmona presenta para aspirar al Grado de Doctor por la Universidad Autónoma de Barcelona

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A mi madre y a mi padre,

por su cariño, dedicación y paciencia.

"Lo único necesario para que el mal triunfe
es que las buenas personas no hagan nada"

Edmund Burke

"Que sea difícil no implica que sea imposible,
que sea imposible no implica que vaya a rendirme"

El Chojin

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RESUMEN

Estudiar la dinámica poblacional de las especies vegetales y el efecto de los distintos factores bióticos y abióticos en su reproducción es esencial para conocer y entender la biología reproductiva de las plantas. Este conocimiento tiene especial relevancia desde el punto de vista de la conservación cuando se centra en especies endémicas y/o amenazadas.

En esta tesis doctoral hemos estudiado en profundidad la reproducción de *Erysimum popovii*, una herbácea endémica del sureste de la Península Ibérica y catalogada como "Casi Amenazada". Se seleccionaron 28 poblaciones a lo largo de todo el área de distribución de la especie, en las que se midieron las distintas variables relacionadas con polinizadores, hábitat y clima. El hecho de abarcar todo el rango de distribución de la especie, ha permitido hacer análisis espaciales a escala poblacional y regional, determinando el estado de la especie y su reproducción a nivel de especie.

En el **Capítulo 1** se analiza cómo el conjunto de los polinizadores, así como otras variables bióticas y abióticas afectan a la reproducción de *E. popovii*. Para ello se realiza un estudio demográfico de la planta analizando los distintos estadios de su ciclo de vida. En el **Capítulo 2** se profundiza en la limitación de polen en *Erysimum popovii*, realizando un experimento de adición de polen y analizando las variables que afectan a dicha limitación, así como su distribución espacial. Además de la limitación por polen, las poblaciones de plantas pueden verse limitadas por el reclutamiento. Este aspecto se aborda en el **Capítulo 3** gracias a un experimento de adición de semillas. Por último, el **Capítulo 4** indaga en el estado de conservación de las poblaciones de *E. popovii*, estudiando las diferencias en la viabilidad de las poblaciones dentro y fuera de las áreas protegidas.

E. popovii mostró diferencias espaciales en las variables reproductivas estimadas a lo largo de su área de distribución. Estas diferencias van acompañadas por diferencias en hábitat, polinizadores, etc. De esta forma, la especie se encuentra con condiciones distintas en las distintas localidades que habita lo que genera un mosaico geográfico en las poblaciones de *E. popovii*. Esto podría indicarnos un comportamiento metapoblacional de la especie. Una de las variables que más favorece la reproducción de *E. popovii* ha sido la presencia de Bombílidos. Este resultado sugiere que estos dípteros son un grupo de polinizadores altamente eficaces como vectores polínicos de esta planta. Por el contrario, la densidad de especies florales acompañantes afecta de forma negativa a la fructificación de *E. popovii*, lo que indicaría una posible competencia interespecífica. La producción de semillas de la mayoría de las poblaciones está limitada por polen. Además, algunas poblaciones están limitadas por la disponibilidad de micrositios, la producción de semillas o su dispersión. Se detecta un cuello de botella muy importante en el paso de plántula a adulto. En general, existe una gran variación espacial en estos tipos de limitación, como queda patente y esta variación no muestra un patrón geográfico claro.

Finalmente se hacen algunas recomendaciones para mejorar la conservación de la especie. La principal recomendación es el establecimiento de zonas de exclusión de herbivoría, que actúen como fuentes de propágulos que favorezcan la dinámica metapoblacional de la especie. Además se aconsejan otras medidas como búsqueda de nuevas poblaciones, la dispersión manual de semillas y el estudio filogeográfico de la especie.

SUMMARY

The study of plant population dynamics and the effects of biotic and abiotic factors on reproductive success is essential to understand plant reproductive biology. This is especially relevant from the standpoint of conservation of endemic and/or endangered species.

In this PhD thesis we have studied the reproductive success of *Erysimum popovii*, a "near-threatened" endemic herb from SE Spain. We selected 28 study sites throughout the entire distribution area of the species. At each site we measured several *Erysimum popovii* populational variables, as well as variables related to pollinator composition, habitat and climate. Because our study encompasses the entire distribution range of the species, we have been able to analyze our results and establish the status of the species both at the local and regional levels.

Chapter 1 addresses the effect of pollinator composition and other biotic and abiotic factors on the reproductive success of *Erysimum popovii.* To do this, we conducted a demographic study covering the different phases of the life cycle. In **Chapter 2** we study pollen limitation. We conducted a pollen supplementation experiment and analyzed the spatial distribution and the factors affecting pollen limitation. In addition to pollen limitation, plant populations may be limited by their ability to recruit. We address this issue in **Chapter 3**, in which a seed supplementation experiment was conducted. Finally, **Chapter 4** analyses the conservation status of *Erysimum popovii* populations in protected *versus* unprotected areas.

Erysimum popovii shows dramatic differences across its distribution range in the reproductive variables measured. These differences are accompanied by differences in pollinator composition and habitat factors. Therefore, different populations encounter different local conditions, thus generating a geographic mosaic and suggesting a metapopulation structure. One of the main variables associated to reproductive success in *Erysimum popovii* is visitation by Beeflies. This result suggests that these dipterans are highly effective pollinators of this species. On the other hand, high densities of co-flowering plants have a negative effect on *Erysimum popovii* fruit set, indicating possible interspecific competition effects. Seed production is pollen-limited in most of the *Erysimum popovii* populations. In addition, some populations are limited by the availability of microsites, seed production and/or seed dispersal. We found an important reproductive bottleneck in the seedling to adult transition. The spatial variability of these various types of limitation is very high and does not show any clear geographical pattern.

The main recommendation to improve the conservation status of the species is the establishment of areas of exclusion of herbivores. These areas may act as sources of propagules enhancing the metapopulational dynamics of the species. We also recommend the search of new populations, seed supplementation of certain populations and the phylogeographic study of the species.

INTRODUCCIÓN GENERAL

LA POLINIZACIÓN EN LAS INTERACCIONES ANIMAL-PLANTA

Las interacciones entre animales y plantas juegan un papel clave en la estructura de las comunidades y determinan su distribución espacial (Boucher 1985; Begon 1999). Algunas de las interacciones que se dan entre plantas y animales pueden considerarse mutualistas, cuando plantas y animales se proporcionan beneficio mutuo. Esto produce un incremento en las tasas de natalidad, una disminución en las tasas de mortalidad y/o una mayor capacidad de carga (Begon 1999). Las dos formas más comunes de mutualismo animal-planta son la polinización y la dispersión de semillas, jugando ambas un papel fundamental en la reproducción y el establecimiento de las poblaciones de plantas y de los animales con los que interaccionan (Burd 1994; Jordano et al. 2003). La polinización mediada por insectos es el sistema de polinización más extendido, ya que casi un 70% de las especies de plantas se reproducen mediante este sistema (Kearns & Inouye 1997, Richards 1997). Por tanto, no es de extrañar que la biología de la polinización haya despertado el interés de muchos investigadores y hayan sido numerosas las investigaciones que han realizado en este campo.

Además, la polinización es uno de los factores que más intensamente afecta a la distribución espacial de las especies vegetales con flores, ya que la variación espacial del conjunto de sus polinizadores puede determinar el éxito reproductivo de las plantas en distintas localizaciones. Según se ha estudiado, un elevado porcentaje de plantas presentan limitación por polen (Burd 1994; Ashman et al. 2004; Knight et al. 2005), es decir, su capacidad reproductiva se encuentra limitada por la cantidad y calidad de polen que reciben. Por tanto, la variación espacial y temporal de los polinizadores puede afectar a la dinámica y distribución de las plantas. De hecho, muchos estudios han mostrado variación espacial en la abundancia y composición de los polinizadores y el efecto que esto ejerce sobre las poblaciones de plantas a las que polinizan (Price et al. 2005; Gómez & Zamora 2006; Ollerton et al. 2006; Gómez et. al. 2007). Esta variabilidad espacial puede generar mosaicos de composición de polinizadores en una región (Gómez et al. 2009). Son numerosos los factores que pueden determinar las diferencias en dicha estructura en mosaico, afectando a la abundancia, diversidad y composición de la comunidad de polinizadores en un área determinada. Entre los factores abióticos destacan la temperatura y la altitud (Arroyo et al. 1985, Blionis & Vokou 2001; Rahbek 2005), la litología del terreno (Potts et al. 2005), la pendiente y la disponibilidad de substratos de nidificación (Cane 1991, Potts et al. 2005, Grundel et al. 2010). Entre los factores bióticos destacan la diversidad de la flora (Tepedino & Stanton 1981; Gathman et al. 1994; Croxton et al. 2005), la densidad de plantas entomófilas (Banaszak 1996, Roulston y Goodell 2011), la presencia de parásitos (Roulston & Goodell 2011) y la abundancia de hábitat naturales (Steffan-Dewender et al.2002).

Por tanto, saber la identidad y la efectividad de los polinizadores de una determinada planta, la diversidad del conjunto de éstos y la tasa con la que los polinizadores contactan con la planta de forma efectiva, se hace imprescindible para conocer de forma precisa el éxito reproductivo de una especie, así como comprender su distribución y predecir su comportamiento ante determinados escenarios (Klein et al. 2003; Gómez et al. 2007).

IMPORTANCIA DE LA POLINIZACIÓN PARA LA CONSERVACIÓN DE ESPECIES VEGETALES

Los planes de conservación para una especie que se encuentra en peligro, generalmente, requieren la conservación de la mayor cantidad posible de individuos en un área determinada (Curry-Lindahl 1982). Siempre ha de mantenerse por encima de la Población Mínima Viable, que según Shaffer (1981) es la población más pequeña de una especie, que estando aislada, tiene una posibilidad de sobrevivir del 99% durante 1000 años. En el caso de especies vegetales polinizadas por animales, la PMV puede depender de forma directa de los polinizadores de la especie y de forma indirecta de las variables bióticas y abióticas que afecten a dicha interacción. En la medida en que estas variables pueden variar de unas zonas a otras, también habrán de hacerlo los planes y medidas que se tomen para la conservación de dichas especies vegetales.

En el caso de las especies endémicas y en peligro, los análisis demográficos son de especial importancia, puesto que en muchos casos se trata de poblaciones pequeñas y aisladas (Primack & Ros 2002; Groom et al. 2006). Las poblaciones pequeñas están más expuestas a pérdidas de la variabilidad genética, relacionada con depresiones endogámicas y pérdida de la flexibilidad evolutiva. Además, dado su pequeño tamaño pueden sufrir fluctuaciones poblacionales relacionadas con sus propias dinámicas poblacionales o con variaciones ambientales o catástrofes que generen vórtices de extinción que llevarían a la desaparición de la población.

Además, pequeñas alteraciones en las relaciones de estas plantas con sus polinizadores pueden dificultar la recepción del polen, con consecuencias dramáticas para su conservación (Tepedino et al. 1997). Actualmente se esta observando una disminución en las poblaciones de polinizadores, fenómeno que se conoce como crisis de polinización (Kearns et al 1998; Ghazoul 2005; Beisjmier et al 2006). Esto podría poner en riesgo las interacciones planta-polinizador (Kearns et al 1998; Kremen C & Ricketts T. 2000), lo que a su vez podría poner en riesgo a las especies florales, especialmente las endémicas y en peligro, como se ha mencionado anteriormente. Además, el cambio climático podría empeorar esta situación, alterando los periodos de floración, los ciclos de vida de los insectos, etc. (Memmott et al. 2007, Hegland et al. 2009). Por lo tanto, para el establecimiento de eficaces planes de recuperación o conservación de especies florales se hace imprescindible el estudio del conjunto de sus polinizadores y la comprensión de las interacciones que se producen.

Además es importante tener en cuenta que en muchas ocasiones, las poblaciones de plantas no se comportan como poblaciones cerradas y estables, sino que responden a un mosaico cambiante de poblaciones temporales conectadas por migración, lo que se conoce como metapoblaciones (Bonet et al. 2005; Rozenfeld et al. 2008). En estos casos, numerosas poblaciones son pasajeras, pudiendo cambiar drásticamente de unas generaciones a otras, estableciendo áreas satélite con poblaciones fluctuantes. Estas áreas satélite se mantienen y generan gracias a núcleos estables que proporcionan individuos que migran a nuevas zonas o recolonizan zonas donde la especie se ha extinguido localmente (Hanski & Simberloff 1997; Primack & Ros 2002). Cuando una especie responde a un modelo de metapoblaciones, la fragmentación de su área de campeo suele tener unos efectos dramáticos para su conservación al disminuir la conectividad y reducir la probabilidad de recolonización por migración. Por lo tanto, es imprescindible conocer la dinámica de las poblaciones con la que se trabaja así como la conectividad entre ellas, si queremos llevar a cabo una gestión adecuada y eficaz.

ESPECIE DE ESTUDIO, DISTRIBUCIÓN GEOGRÁFICA Y ESTATUS DE CONSERVACIÓN

Los estudios que se desarrollan en los sucesivos capítulos de la presente tesis fueron realizados utilizando como sistema de estudio la crucífera *Erysimum popovii* Rothm. Esta planta de la familia Brassicaceae es perenne de vida corta. Se reproduce a partir del segundo año, aunque en algunas excepciones se han observado individuos capaces de reproducirse al año de vida (observaciones en el invernadero). Presenta un número variable de tallos floríferos (1-8) erectos, simples, esparcidos o densamente poblados (Blanca et al. 1992, Blanca et al. 2009). La altura de los tallos floríferos puede variar entre los 10 y 50 cm. aunque excepcionalmente pueden verse ejemplares de mayor altura (observaciones en el campo). La floración ocurre entre los meses de abril y mayo, la planta produce flores violáceas cuya cantidad puede variar entre menos de una decena y más de un centenar. Las flores se presentan en racimos terminales simples. Los frutos maduran en el mes de agosto y se dispersan de octubre a diciembre mediante mecanismos de barocoria. Como ocurre con otras especies del género *Erysimum* (Gómez 2007), sus tallos, racimos florales y frutos son consumidas por ganado vacuno, ovino y caprino además de otros ungulados. De forma excepcional se han observado frutos de *E. popovii* parasitados por larvas de curculiónidos (posiblemente *Ceutorhynchus chlorophanus*).

Erysimum popovii se distribuye por las Sierras Béticas de Andalucía Oriental, en zonas montañosas de las provincias de Granada (Sierra Nevada, Sierra Arana, Sierra de Loja, Sierra de Cogollos, Sierra de Alhama, Sierra de Tejeda, Sierra de la Peza y El Mencal), Jaén (Sierra Mágina y Sierra de Jabalcuz) y Córdoba (Sierra Horconera). Las zonas muestreadas presentan un clima Mediterráneo continental, caracterizado por inviernos fríos y veranos calurosos, con una marcada sequía estival. La mayoría de las precipitaciones se producen en primavera y otoño. La temperatura media anual varía entre 9,2 y 14,2ºC, encontrándose las mínimas de invierno por debajo de los 0ºC y las máximas de verano próximas a los 40º C. Algunas de las zonas de estudio se encuentran dentro de parques naturales, en concreto el Parque Natural de Sierra Nevada (Granada), Parque Natural de Sierra Mágina (Jaén) y Parque Natural de las Sierras Subbéticas (Córdoba). Durante 2007 y 2008 se revisaron las zonas donde había citas de la presencia de la especie, para localizar un conjunto suficiente de poblaciones para llevar a cabo las investigaciones. Se encontraron 31 poblaciones de *E. popovii* a lo largo de su área de distribución: 2 en Sierra Nevada, 7 en la Sierra de la Peza, 6 en Sierra Arana, 4 en la Sierra del Mencal, en la provincia de Granada; 7 en la Sierra de Jabalcuz, 4 en Sierra Mágina, en la provincia de Jaén; y 1 en la Sierra de la Horconera, en la provincia de Córdoba (Figura 1). De las 31 poblaciones encontradas, 29 fueron seleccionadas para las investigaciones, ya que una población se perdió como consecuencia de trabajos silvícolas llevados a cabo en la Sierra de Jabalcuz, y otra población fue desestimada por encontrarse en un lugar de difícil acceso. También desestimamos el usar poblaciones de la Sierra de Cazorla y la Sierra del Pozo, al estar muy solapadas con poblaciones de *E. cazorlense* y *E. baeticum*, lo que podría haber afectado a nuestros resultados, ya que las especies son muy difíciles de distinguir. No pudimos encontrar poblaciones ni en la Sierra de Loja ni en la Sierra de Tejeda, después de una intensa búsqueda. Esto puede deberse a que en estas zonas, la especie haya sufrido un proceso de extinción local, ya que las citas consultadas eran muy antiguas. Sin embargo, hemos encontrado poblaciones en zonas donde no se tenía registrada la presencia de la especie, como Sierra Nevada.

Figura 1. *Distribución de los grupos de poblaciones de Erysimum popovii utilizadas en las investigaciones de la tesis.*

En estas 29 poblaciones se caracterizaron aquellas variables que pudiesen afectar a la relación entre los polinizadores y la planta, en base a investigaciones previas realizadas y a la bibliografía consultada. Se determinó el tamaño y densidad de las poblaciones, la estructura del hábitat, composición de la comunidad floral, el conjunto de los polinizadores de *E. popovii*, tipos de hábitats colindantes y diversas variables topográficas y climatológicas.

Es importante señalar que la región mediterránea, donde se ha desarrollado esta tesis doctoral, posee unas excelentes condiciones para la realización de investigaciones sobre las interacciones polinizador-planta y sus dinámicas temporales. Esto se debe a que, en dicha región, la floración está muy restringida temporalmente, así como el periodo de actividad de los polinizadores, produciéndose entre la primavera y los primeros meses de verano. La floración y las interacciones polinizador-planta suelen estar muy afectada por factores abióticos que fluctúan y varían entre zonas y años (Herrera 1986, Arroyo 1990). Los ecosistemas mediterráneos presentan una enorme diversidad de especies, encontrándose entre los sistemas más diversos del mundo (Mittermeier et al. 1998; Blondel & Aronson 1999). Además, las zonas mediterráneas se caracterizan por una fuerte heterogeneidad espacial, como consecuencia de una acentuada estacionalidad que genera fuertes contrastes de humedad y temperatura. La sequía estival es uno de los factores más limitantes y determinantes para las comunidades vegetales. A esto hay que sumarle la complejidad orográfica, lo que sin duda, mejora sus cualidades para la realización de este tipo de investigaciones, ya que permiten encontrar distintos escenarios en zonas próximas, así como cambio de condiciones hídricas y climatológicas entre años para una misma zona, pudiendo por tanto realizar complejos estudios comparativos.

OBJETIVOS Y ESTRUCTURA EN CAPÍTULOS

El objetivo principal de esta tesis es estudiar el éxito reproductivo y las interacciones entre *Erysimum popovii* y sus polinizadores y determinar sus implicaciones para la conservación de la especie. Aunque los resultados son solo referentes a *E. popovii* el planteamiento del estudio y la metodología pueden aplicarse a otras especies en estatus de conservación similares. El objetivo principal se descompone en distintos objetivos parciales que son abordados en los distintos capítulos.

En el **Capítulo 1**, se analiza cómo el conjunto de los polinizadores y sus componentes de abundancia, diversidad y composición afectan a la reproducción de *E. popovii*. Para ello se realiza un estudio demográfico de la planta analizando los distintos estadios de su ciclo de vida. Además se analiza cómo la composición del hábitat y otras variables poblacionales pueden afectar a la reproducción de la planta. Todo esto nos permite desarrollar un amplio conocimiento sobre la reproducción de *E. popovii* y estudiar su variabilidad entre las distintas poblaciones y regiones donde la especie está presente.

En el **Capítulo 2** se lleva a cabo un estudio sobre la limitación de polen en *Erysimum popovii*, realizando un experimento de adición de polen. Específicamente se analiza la variación geográfica de la limitación de polen a lo largo de toda el área de distribución de la especie y su relación con varios factores ambientales y con la composición de polinizadores. También se analiza si los efectos de la limitación por polen son más intensos a nivel pre o post-dispersivo. La existencia de limitación por polen permite justificar la posible relación existente entre la presencia de determinados polinizadores en una zona y el éxito reproductivo a nivel local.

En el **Capítulo 3** se exponen y analizan los resultados de un experimento de adición de semillas, cuyo objetivo era estudiar si las poblaciones están limitadas por la producción de semillas, por la dispersión de las mismas o por la disponibilidad de micrositios para su germinación. Ello nos permite conocer qué factores limitan la tasa de reclutamiento en cada estadio a lo largo del ciclo de vida completo.

Finalmente, en el **Capítulo 4**, se indaga en el estado de conservación de las poblaciones de *E. popovii*. Además se estudian las diferencias en la viabilidad de las poblaciones dentro y fuera de las áreas protegidas, se analiza el efecto del daño por ungulados a la conservación de la planta y se sugieren medidas concretas para la mejora de la conservación de la especie.

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CAPÍTULO 1

REPRODUCTIVE SUCCESS OF THE NARROW ENDEMIC *ERYSIMUM POPOVII*: GEOGRAPHICAL VARIATION AND THE EFFECTS OF POLLINATOR COMPOSITION AND ABIOTIC CONDITIONS

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Reproductive success of the narrow endemic *Erysimum popovii*: Geographical variation and the effects of pollinator composition and abiotic conditions.

ABSTRACT

Geographic variation in reproductive outcome determines plant distribution. In this study, we investigate the geographic structure and the factors affecting reproductive success throughout the life cycle of the near threatened crucifer *Erysimum popovii* across its entire distribution range. We worked in 21 populations, in which we measured fruit set, seed set, seed weight, seed germination in the laboratory, germination time, seedling emergence in the field, seedling survival and fecundity. We also sampled the pollinator assemblages visiting *Erysimum popovii* in each site, as well as some populational characteristics (population size and density, flower density of *E. popovii* and of other co-occurring species, and rainfall). Germination success in the laboratory was very high (range: 0.56-0.98), but seedling emergence in the field was low (0.005-0.32). Beefly visitation rates were positively related to seedling emergence, whereas visitation rates by low-quality pollinators were associated to decreased fruit set and germination time. Populations in sites with high density of co-occurring flowers produced fewer fruits. Most variables related to reproductive outcome varied widely across populations, but this variation did not show a defined regional structure. The low seedling survival may constitute a bottleneck for the recruitment of this species. Overall, less than 0.2 % of the ovules produced turned into reproductive individuals. Our results suggest a metapopulation structure for *Erysimum popovii*.

KEYWORDS: *Reproduction · Erysimum popovii · Fitness · Seed set · Fruit set · Seed weight · Germination time · Germination · Seedling*

INTRODUCTION

The study of factors determining plant reproduction is crucial to understand species distribution. Most studies addressing this issue focus on demographic analyses, and include various phases of the plant's life cycle such as fruit set, seed production and seed germination (e.g. Menges 1991; Lamont et al. 1993; Widén 1993; Menges & Gordon 1996; Roll et al. 1997; Colas et al. 2001; Rebollo et al. 2001; Spligerand Chang 2008; Fayolle et al. 2009; Metz et al. 2010; Wirth et al. 2011). Overall, limitation of reproduction in pre-dispersal phases (fruit set and seed production) may be determined by different factors such as the genetic constitution of the mother plant (Anderson 1993), pollen limitation (Ashman et al. 2004; Knight et al. 2005; Aizen & Harder 2007; Fernández et al. 2012), ovule abortion (Stephenson 1981; Stephenson & Winsor 1986), resource limitation (Bloom et al. 1985), and predation (Grieg 1993). Fruit set has often been found to be low in many plants (Collins & Rebelo 1987; Ayre & Whelan 1989; James et al. 1994; Guitián et al. 1996), supporting the view that flowers represent a reservoir of ovules that can be used when resources are plentiful, thus providing an adaptive response to stochastic environments. Instead, under sub-optimal conditions, plants may save resources by discarding flowers or fruits at the beginning of their development (Ayre & Whelan 1989). In addition to fruit set, final seed yield, may also be limited by low seed set within fruits (ratio of ripe seeds to ovules). Limitation of reproduction in the post-dispersal phase (seed germination) has commonly been linked to plant population size (Menges 1991), density of conspecific and heterospecific plants (Fenner & Kitajima 1999), abiotic conditions such as precipitation regime (Philippi 1993; Clauss & Venable 2000; Venable 2007), and seed quality (Winn 1985; Burke & Grime 1996). Two of the most widely used estimators of seed quality are seed weight and germination time. Seed weight affects many processes of the reproductive cycle, including seed germination (Schall 1980; Hendrix 1984; Naylor 1993), dormancy period (Stamp 1990; Fenner & Thompson 2004), resistance to competition (Houssard & Escarre 1991; Turnbull et al. 2004), seed dispersal (Augspurger & Franson 1986; Fenner & Thompson 2004) and seedling growth and recruitment (Lloret et al. 1999; Walters & Reich 2000; Coomes & Grubb 2003). Germination time, on the other hand, may be specially determinant in annual and herbaceous plant species at the community level via competitive dominance of the early germinating individuals. Very short delays in emergence time can be magnified in the long term, with strong effects on final biomass and reproductive output (Kalisz 1986; Weiner & Thomas 1986).

Therefore, different life cycle phases are affected by different extrinsic biotic and abiotic factors, which in addition, may vary spatially at different scales (Mc Geoch & Price 2005; Anderson & Johnson 2008; Nattero et al. 2011). That is, the relative contribution of the different phases of the life cycle to the plant's reproductive output may vary spatially depending on the locally prevailing biotic interactions and abiotic conditions (Pavlik et al. 1993; Edwards and Crawley 1999; Schupp et al. 2002; Stevens et al. 2004; Rautio et al. 2005; Kim & Donohue 2011). Pollinator abundance, composition and diversity have often been related to plant reproductive success (Waser 1978; Motten et al. 1981; Steffan-Dewenter et al. 2001; Waites & Agren 2004; Cosacov et al. 2008; González-Varo et al. 2009; Gómez et al. 2010). Pollen limitation is often associated to low pollinator abundance (Cosacov et al. 2008; González-Varo et al. 2009; Gómez et al. 2010), and different pollinator species usually differ in pollinating effectiveness and may thus have different effects on plant fitness (Klein et al. 2002; Gómez et al. 2007; Perfectti et al. 2009). Additionally, some habitat characteristics such as plant population size, habitat fragmentation, co-occurring flower composition, and altitude can influence plant reproduction either directly or indirectly via their effect on biotic interactions (Totland 2001; González-Varo et al. 2009; Jakobsson et al. 2009; Kim & Donohue 2011). Understanding how these biotic and abiotic variables affect different phases of the reproductive cycle is essential to interpret the reproduction dynamics, geographical structure and conservation status of a plant species (Menges 1990; Colas et al. 2001; Metz et al. 2010).

In this study, we explore the geographic variation of reproductive success and recruitment, as well as their relationship with local pollinator assemblages and abiotic conditions in *Erysimum popovii*, a narrow endemic Brassicaceae. Our objectives are: 1) to study the different phases of the reproductive cycle and evaluate their relative contribution to recruitment; 2) to analyze the geographic structure of the reproductive output across the entire distribution range of the species; 3) to measure the effect of pollinator diversity, abundance and composition on the various phases of the reproductive cycle; and 4) to measure the effect of *E. popovii* population characteristics and habitat composition on the various phases of the reproductive cycle.

MATERIALS AND METHODS

STUDY SYSTEM

Erysimum popovii Rothm. (Brassicaceae) is a narrow endemic species from southeastern Spain, categorized as “Near Threatened” in the Red List of Andalusian Vascular Flora (Cabezudo et al. 2005). The species shows a scattered distribution across the Baetic mountain ranges from 900 to 2000 m. a.s.l., inhabiting rocky areas and shrubland gaps. It is a biennial to perennial monocarpic herb, producing from a few to several hundred flowers on a variable number of reproductive stalks. Flowers are hermaphroditic, slightly protandrous, with four bright purple petals and a tetradynamous androecium (four long and two short stamens) (Blanca et al. 1992; Blanca et al. 2009). Like other *Erysimum* species (Gómez 2005b), *E. popovii* is partially self-compatible, but requires pollen vectors for full seed set (Author's unpublished data).

The study was conducted in 21 *Erysimum popovii* populations located in the Granada and Jaén provinces, spanning the entire geographic range of the species (Table 1). These populations are distributed accross 6 regions (Mencal, Sierra Arana, Sierra Nevada, La Peza, Sierra de Jabalcuz, Sierra Mágina). Once field work was completed, two populations from the Mencal region (Ep01 and

Ep19, Table 1) were provisionally reassigned by taxonomists to the related taxon *E. baeticum bastetanum*. However, and because taxonomic relationships are far from being resolved in *Erysimum* species from SE Spain, we decided to keep these two populations in our study. Geological substrates are mainly limestone and dolomites (Vera 2004). The overall area has a characteristic Mediterranean climate type, with cool wet winters and warm dry summers. The annual average rainfall ranges between ca. 500 and 1000 mm, annual mean temperatures between 9.2 and 14.2ºC.

REPRODUCTIVE OUTPUT

We measured several variables related to reproductive success: 1) Fruit set. The proportion of flowers that produced fruits, estimated in the field in 2008 and 2009 in 30 randomly-selected plants per population. 2) Seed set. The number of ripe seeds per ovule. Four ripe fruits in each of the 30 above-mentioned plants were taken to the laboratory and the number of seeds was counted under a magnifying lens. We were able to distinguish aborted seeds from unfertilized ovules because, as in many other crucifers, *Erysimum* aborted seeds are dark brown, with shriveled cotyledons and embryo (Gómez et al. 2010), whereas virgin ovules are creamy white, lanceolate in shape and of a far smaller size. 3) Seed weight (in mg). We weighted three samples of ten seeds per population and averaged the three weights. Reported values express weight per 100 seeds in each population. 4) Seed germination. We recorded the germination of 125 randomly-selected ripe seeds per population under laboratory conditions. Seeds were distributed in 5 pasteurized 90-mm Petri dishes (25 seeds per dish) containing a layer of small glass balls (ca. 3 mm diameter) with a filter paper disk on top. Afterwards, we added 10 ml of distilled water. To avoid mold propagation, all seeds were previously disinfected with 5% sodium hypochlorite solution for 3 minutes. Dishes were placed in a germination chamber (day/night cycle 16h/8h; temperature 16ºC/24ºC ± 2ºC). Seeds that produced a radicle protruding 1 mm or more were scored as germinating. Germination checks were conducted three times per week, during 60 days. After each check, dishes were randomly shuffled within the chamber to avoid potential position effects. 5) Germination time. The time needed to reach 50% of total germination (T50, in days) (Kos & Poschlod 2010). We averaged the germination times in each of the five above-mentioned Petri dishes. Low T50 values denote high germinating speeds. 6) Seedling emergence. This parameter was determined experimentally under field conditions. At the end of winter 2009, 200 randomly selected seeds were sown per population distributed in 10 replicates of 20 seeds each. Once a month we counted the number of emerged seedlings. 7) Seedling survival. This parameter was also determined under field conditions. In November of 2009 we conducted a seed supplementation experiment. Seeds were sown in 0.25 m^2 permanent quadrats, replicated 10 times across each population. The number of seeds used per replicate was 10 times the estimated seed density of each population (obtained from data on population size, fruit set and seed set in each population). We used such a high seed density to ensure a sufficient number of juveniles in spite of the low rate of seedling emergence, and to account for the aggregated seed distribution observed in natural populations of *E. popovii*. From December 2010 to May 2011 we counted the juveniles (pre-adult rosettes) growing in the quadrats. 8) Fecundity. We quantified fecundity as the production of juveniles per reproductive adult (n=30), and used the average as an inclusive estimate of the plant reproductive output in each population. The transition from juvenile to adult is near 1, as almost all juveniles become adults (field and greenhouse observations).

Population Code	Region (Province)	Coordinates (UTM)	Elevation (m a.s.l.)	Population Size (reproductive individuals)	Plant Density (individuals/ m^2)	*E. popovii* flowers (flowers/population)	Density of *E. popovii* flowers (flowers/m^2)	Density of co-occurring flowers (flowers/m^2)	Mean annual rainfall (mm)
Ep01	Mencal (Granada)	30S 0487836/ 4155063	982	600	0.015	3320	0.85	15.63	493.5
Ep02	S. Arana (Granada)	30S 0451977 /4128858	1255	340	0.011	3264	0.33	13.49	579.6
Ep03	S. Nevada (Granada)	30S 0456751 /4109531	1596	280	0.037	784	0.88	23.34	510.2
Ep04	La Peza (Granada)	30S 0467701 /4118300	1503	300	0.073	1340	1.30	25.99	577.0
Ep05	S. Jabalcuz (Jaen)	30S 0425545 /4177799	1321	420	0.197	2502	7.79	17.33	725.6
Ep07	S Mágina (Jaen)	30S 0453888 /4176684	2000	500	0.135	2517	2.68	31.81	549.6
Ep08	S. Jabalcuz (Jaen)	30S 0425519 4177931	1324	400	0.173	1323	4.31	7.74	726.7
Ep09	La Peza (Granada)	30S 0472834 4119460	1693	130	0.085	637	2.18	16.55	609.1
Ep10	S. Jabalcuz (Jaen)	30S 0424122 4177754	1060	450	0.064	5430	3.94	19.28	737.3
Ep13	S. Arana (Granada)	30S 0454337 4131901	1738	370	0.087	2676	2.73	228.36	642.4
Ep14	S. Jabalcuz (Jaen)	30S 0426894 4180210	970	120	0.025	1436	2.19	8.82	689.0
Ep15	S. Jabalcuz (Jaen)	30S 0425148 4179387	1080	650	0.062	8060	4.51	6.13	648.0
Ep16	S. Jabalcuz (Jaen)	30S 0425152 4179487	1060	150	0.038	1965	2.01	1.97	640.5
Ep19	Mencal (Granada)	30S 0483826 4151106	1188	420	0.246	1470	6.84	16.21	496.7
Ep20	S. Arana (Granada)	30S 0451048 4130531	1270	350	0.069	1435	2.26	30.63	640.6
Ep21	S. Arana (Granada)	30S 0452839 4131133	1498	280	0.113	1820	4.35	24.47	675.5
Ep22	S. Arana (Granada)	30S 0453676 4128236	1380	620	0.069	3968	2.32	66.38	569.7
Ep23	La Peza (Granada)	30S 0472949 4119278	1720	750	0.556	5650	18.56	28.23	602.6
Ep27	S. Nevada (Granada)	30S 0457430 4109448	1486	340	0.176	1530	3.22	23.56	540.6
Ep28	S Mágina (Jaen)	30S 0455789 4176686	1661	1650	0.193	5775	5.42	61.62	559.4
Ep30	S Mágina (Jaen)	30S 0453436 4176812	1898	2050	0.133	13598	2.03	30.47	582.7

TABLE 1. Location and characteristics of the 21 E. popovii study populations.

POLLINATOR ASSEMBLAGES

In the springs of 2008 and 2009 we recorded the identity and abundance of pollinators visiting the flowers of *E. popovii* in each population. Surveys were carried out from 11:00 am to 17:00 pm (GMT +2) on several days throughout the flowering period. Observations were done on groups of plants comprising about 100 flowers. Total survey time per population ranged between 190 and 795 minutes, depending on pollinator abundance. We tried to reach a minimum of 200 pollinators per population, a sampling effort adequate to describe pollinator assemblages in other *Erysimum* species (Gómez et al. 2007, 2014). We only recorded those insects contacting the sexual parts of the flower, thus acting as legitimate pollinators. We observed a total of 5,169 insects, ranging from 193 to 331 per population. Specimens (n=486) of most morphospecies were captured for later identification in the laboratory.

We estimated pollinator diversity using the Hurlbert's PIE index, which measures the probability that two randomly sampled individuals from the community belong to different species. This index was computed using EcoSim 7 (Gotelli & Entsminger 2009). In addition, we quantified the visitation rate, measured as the number of visits per hour of survey, of four pollinator functional groups (following Gómez et al. 2007, 2008): 1) Large bees: > 10 mm in body length; 2) Small bees: < 10 mm in body length; 3) Beeflies: long-tongued Bombyliidae; 4) Low Quality pollinators: including ants, beetles, hoverflies and other minor groups previously shown to have low pollinating efficiency on *Erysimum* (Fernández and Gómez 2012).

PLANT POPULATION CHARACTERISTICS

We characterized each *E. popovii* population with the following variables: 1) Population size. Number of reproductive individuals; 2) Population density. Number of individuals per square meter; 3) *E. popovii* flowers. Estimated number of *E. popovii* flowers per population, based on 30 randomly-selected plants per population; 4) Co-occurring flower density, measured along three transects of 10 x 2 meters in each population, in which we counted the number of flowers of all species blooming at the same time as *E. popovii*; and 5) Mean annual rainfall (in mm), obtained from the Digital Climatic Atlas of the Iberian Peninsula (Ninyerola et al. 2005).

DATA ANALYSIS

We used Markov chain Monte Carlo Multivariate Generalised Linear Mixed Models to test for differences among populations and regions in plant reproductive output (MCMCglmm package, R Development Core Team 2008). We tested each variable related to reproductive output separately. Fruit set, seed set, germination and seedling emergence and survival were fitted to binomial distributions, whereas germination time and seed weight were fitted to Gaussian distributions. We built three models for each reproductive output variable: a first model including only Region as fixed factor, a second model including Population nested into Region as random factor, and a third model including both Population and Region as independent variables. Then, we compared the three models using the deviance information criterion (DIC), and selected the model with lowest DIC value (Hadfield 2010). DIC is a Bayesian analogue of AIC, with a similar justification but wider applicability (Spiegelhalter et al. 2002). Each model was iterated 50000 times.

We tested for spatial structure in our dataset. We explored the spatial autocorrelation of the variables with Moran’s I coefficient. This coefficient measures whether the values of close populations are more similar than expected by chance (Rangel et al. 2010). The number of distance classes was established by the default function of the software SAM v. 4.0 (Rangel et al. 2010) and the significance test was based on 200 permutations.

The relationship between *E. popovii* reproductive output, pollinator assemblage, and population characteristics was explored with a set of models including all possible combinations of independent variables. Since some independent and dependent variables were spatially autocorrelated (Appendices A and B), all models were spatially-explicit. Prior to running these models, we checked for multicollinearity by performing pairwise Pearson correlations amongst variables. Since no strong correlation appeared between variables (Appendices C, D and E), we included all independent variables in the models. We ran two regression models for each reproductive output estimate, one including five pollinator variables (pollinator diversity, and visitation rate of the four above-mentioned pollinator functional groups), and the other including five population variables (population size, population density, abundance of *E. popovii* flowers, density of flowers of co-occurring species, and annual rainfall). All these analyses were performed with SAM v. 4.0 (Rangel et al. 2010).

RESULTS

REPRODUCTIVE OUTPUT

Most reproductive output variables varied widely among populations (Table 2; Appendix F). Number of flowers per individual ranged between 15.2 and 79.5 (mean±SD: 37.6±17.9) (Appendix F). In all populations, more than 50% of the flowers produced fruits (fruit set range: 0.50-0.74, mean±SD: 0.67±0.14) (Table 2). The number of ovules per flower ranged between 12 and 39 (Appendix F). Of these, 2.5% aborted, 23.5% were not fertilized, and the rest (74% seed set) produced seeds. Seed set ranged between 61 and 83% (Table 2). Seed weight per 100 seeds ranged between 0.29 and 0.73 gr (mean±SD: 0.5±0.1) (Table 2).

Population	Fruit set	Seed set	Germination success	Seedling emergence	Seedling survival	Seed weight (mg/100seeds)	Germination time (T50)	Fecundity
Ep01	0.671±0.11	0.781±0.13	0.824±0.16	0.170±0.22	0	0.333±0.042	4.904±0.032	0.00
Ep02	0.642±0.14	0.759±0.11	-	0.050±0.06	-	-	-	-
Ep03	0.711±0.08	0.800±0.18	0.840±0.07	0.340±0.16	0.011	0.400±0.02	4.920±0.07	1.69
Ep04	0.702±0.12	0.831±0.09	0.896±0.12	0.315±0.13	0.156	0.403±0.02	4.819±0.01	9.38
Ep05	0.692±0.11	0.783±0.20	0.779±0.16	0.105±0.06	0.007	0.573±0.09	5.744±0.40	0.27
Ep07	0.661±0.16	0.625±0.18	0.928±0.07	0.100±0.1	0.043	0.733±0.07	4.850±0.02	0.23
Ep08	0.649±0.14	0.815±0.09	0.576±0.19	0.045±0.05	0.052	0.387±0.015	5.962±0.39	0.71
Ep09	0.723±0.27	0.808±0.07	0.815±0.09	0.045±0.05	0.174	0.481±0.07	4.950±0.04	2.22
Ep10	0.684±0.09	0.684±0.16	0.824±0.10	0.105±0.14	0.028	0.637±0.10	5.161±0.08	1.33
Ep13	0.500±0.12	0.725±0.19	0.838±0.08	-	0.338	0.563±0.08	9.303±0.41	-
Ep14	0.720±0.11	0.692±0.14	0.960±0.05	0.225±0.10	0.007	0.547±0.15	5.931±0.88	1.78
Ep15	0.721±0.07	0.819±0.10	0.564±0.15	0.130±0.10	0.009	0.487±0.18	5.261±0.15	1.24
Ep16	0.687±0.10	0.697±0.17	0.687±0.08	0.260±0.13	0.042	0.369±0.06	5.453±0.42	6.39
Ep19	0.671±0.13	0.701±0.13	0.824±0.08	0.105±0.10	0.004	0.360±0.05	5.449±0.13	0.13
Ep20	0.739±0.09	0.676±0.16	0.904±0.09	0.206±0.13	0.037	0.623±0.11	5.614±0.44	2.49
Ep21	0.658±0.10	0.766±0.13	0.976±0.04	0.115±0.10	0.023	0.523±0.01	4.892±0.05	1.02
Ep22	0.550±0.19	0.607±0.18	-	0.110±0.09	-	-	-	-
Ep23	0.632±0.12	0.786±0.10	0.666±0.12	0.175±0.12	0.005	0.347±0.03	5.123±0.17	0.24
Ep27	0.635±0.09	0.731±0.13	0.792±0.16	0.255±0.14	0.002	0.290±0.06	5.033±0.09	0.13
Ep28	0.742±0.09	0.767±0.20	0.792±0.10	0.089±0.07	0.018	0.520±0.04	5.911±0.22	0.46
Ep30	0.695±0.13	-	0.952±0.02	0.155±0.11	0.030	0.537±0.06	4.851±0.03	0.60
Total	0.67±0.14	0.74±0.16	0.81±0.15	0.15±0.14	0.058±0.02	0.480±0.13	5.481±0.23	1.68±0.57

TABLE 2. Transition Probabilities of the reproductive output of the 21 Erysimum popovii study populations.

Germination success in the laboratory was very high (mean±SD: 0.81±0.15), but again varied widely across populations (range: 0.56-0.98) (Table 2). Germination time (T50), was approximately 5-6 days in all populations except Ep13 (9 days, Table 2). Seedling emergence in the field was much lower, ranging between 0% and 34% (mean±SD: 14.7%±14.0%) (Table 2), so that seedling production per individual ranged between 5 and 240 (mean±SD: 68.2±72.2) (Appendix F). In addition, seedling survival was low (mean±SD: 0.06±0.02, range: 0-0.34), so that overall fecundity (number of surviving juveniles per reproductive adult was as low as 1.7±0.6) (Table 2). Importantly, fecundity ranged from 0 (no recruitment) to 9.4 (Table 2). On average, only 1.7 of the 839 ovules produced per individual plant resulted into a juvenile with chances of reproducing (Fig. 1).

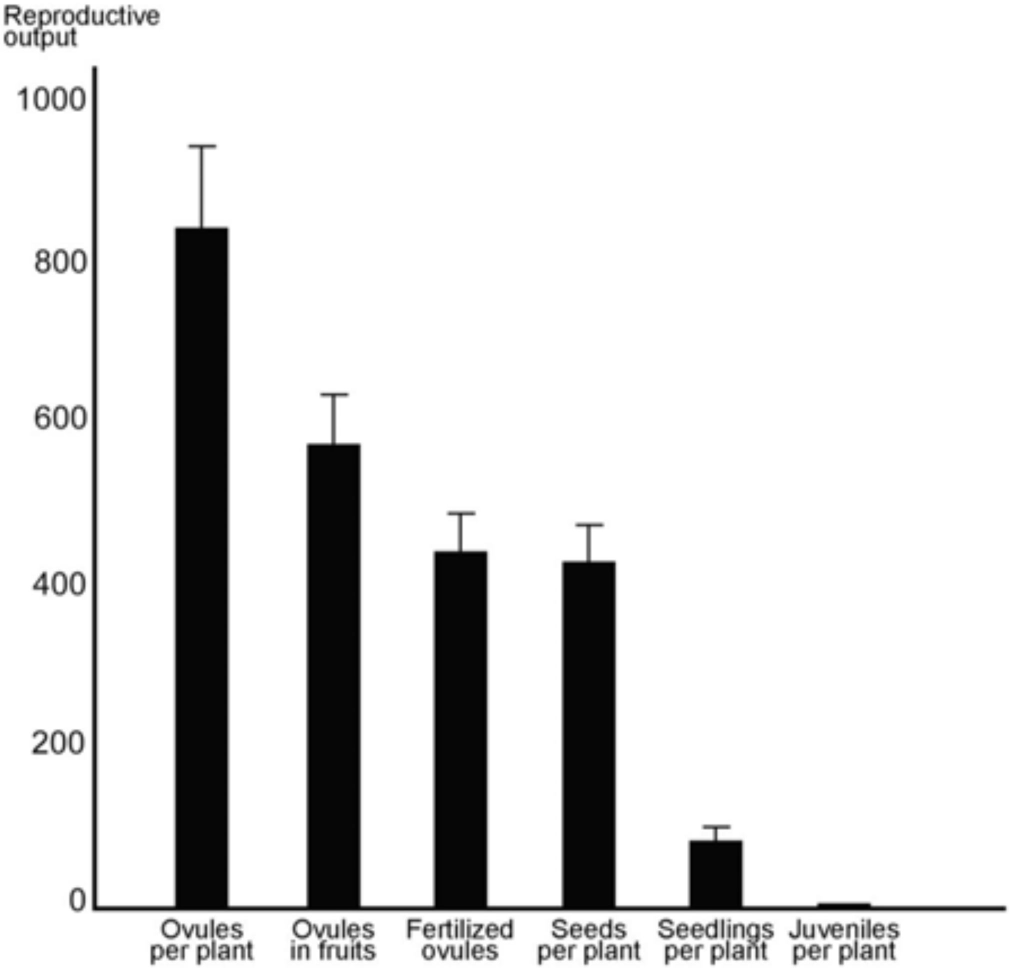

FIGURE 1: Reproductive output (mean ±SE per individual plant) throughout the reproductive cycle of E. popovii.

There were some significant correlations between reproductive output variables. Fruit set was negatively correlated with germination time in the laboratory (r=-0.663, p=0.003), and seed set was negatively correlated with seed weight (r=-0.513, p=0.029) (Appendix E). Seedling emergence was correlated positively with number of seedlings per plant (r=0.566, p=0.014), and negatively with germination time in the laboratory (r=-0.470, p=0.049) (Appendix E). Differences across populations were significant for all reproductive variables, but we found no differences among regions (Table 3). Four of the eight reproductive variables measured showed spatial autocorrelation at one scale or another (Appendix A).

	Model A (region fixed)	Model B (population random)	Model C (region fixed, population random)
Fruit set	27267.61	27233.89	27234.56
Seed set	13698.88	13681.11	13680.85
Seed weight	-87.61	-106.94	-102.70
Germination success	2035.95	2024.19	2023.97
Germination time (T50)	-426.38	-489.87	-489.45
Seedling emergence	3144.87	3128.42	3127.24

TABLE 3. DIC values of models relating several variables of reproductive success to factors population and region. We ran 50000 replicates per model with Markov chain Monte Carlo Sampler.

EFFECT OF POLLINATORS

We recorded 166 insect species visiting the flowers of *Erysimum popovii* in the 21 populations. This assemblage was taxonomically diverse, composed of insects belonging to different functional groups, although large bees and beeflies were the most frequent pollinators, representing 25.9% and 27.6% of the total flower visits, respectively. Pollinator composition varied widely across populations. The number of species recorded per population ranged between 11 and 32, and their diversity (Hurlbert's PIE index), between 0.62 and 0.94 (Appendix G). Large bee visitation ranged between 0.8 and 44.7 visits/hr, and beefly visitation between 0 and 48.2 visits/hr.

	Fruit set			Seed set			Germination success			Seedling emergence			Seedling suvival		
	Coeff ± SE	t	*p* value	Coeff ± SE	t	*p* value	Coeff ± SE	t	*p* value	Coeff ± SE	t	*p* value	Coeff ± SE	t	*p* value
Pollinator variables															
Pollinator diversity (Hurlbert's PIE)	0.017±0.28	0.06	0.953	0.32±0.88	0.36	0.722	-0.54±1.35	-0.40	0.694	0.634±0.37	1.68	0.114	-0.166 ± 0.25	-0.661	0.522
Large bee visitation rate (visits/hour)	<0.001±0.001	0.46	0.654	0.004±0.006	0.62	0.545	0.005±0.01	0.54	0.593	0.003 ± 0.003	1.30	0.214	0.001 ±0.002	0.715	0.49
Small bee visitation rate (visits/hour)	0.002±0.003	0.55	0.588	-0.007±0.01	-0.67	0.512	-0.008±0.01	-0.48	0.637	-0.004±0.004	-0.92	0.371	<0.001 ±0.003	-0.095	0.926
Beefly visitation rate (visits/hour)	<0.001±0.001	-0.81	0.429	-0.004±0.004	-1.05	0.308	0.002±0.005	0.41	0.685	**0.004±0.002**	**2.83**	**0.013**	-0.001 ±<.001	-1.119	0.287
Low-quality pollinator visitation rate (visits/hour)	**-0.005±0.002**	**-2.17**	**0.047**	-0.011±0.01	-1.68	0.114	0.011±0.01	1.08	0.297	-0.001±0.003	-0.41	0.685	<0.001 ±0.003	0.035	0.973
Population variables															
Population size (reproductive individuals)	<0.001±<0.001	7.02	0.061	<0.001±<0.001	-0.79	0.44	<0.001±<0.001	0.908	0.379	<0.001±<0.001	-0.40	0.693	<0.001 ± <0.001	-0.217	0.832
Population density (individuals /m^2)	-0.11±0.083	-1.37	0.193	0.27 ± 0.26	1.05	0.308	0.084± 0.58	0.14	0.888	-0.053 ± 0.17	-0.29	0.769	-0.08 ±0.113	-0.706	0.495
E. popovii flowers (flowers/population)	<0.001±<0.001	-1.48	0.16	<0.001 <0.001	-1.35	0.196	<0.001±<0.001	-0.93	0.368	<0.001±<0.001	0.08	0.938	<0.001 ±<0.001	-0.135	0.895
Density of co-occurring flowers (flowers/m^2)	**<0.001±<0.001**	**-4.61**	**<0.001**	<0.001±<0.001	-0.06	0.948	<0.001±<0.001	-0.06	0.951	<0.001±<0.001	-1.63	0.124	<0.001 ±0.002	0.427	0.678
Mean annual rainfall (l/m^2)	<0.001±<0.001	0.98	0.343	<0.001±<0.001	0.215	0.833	<0.001±<0.001	0.61	0.552	<0.001±<0.001	-1.37	0.192	<0.001 ±<0.001	0.202	0.844

TABLE 4: Coefficient, standard error, t and p values of spatially-explicit models relating several variables of reproductive success with pollinator and population variables separately.

The abundance of low-quality pollinators was negatively related to fruit set and positively related to germination time (Tables 4 and 5). That is, populations with more low-quality pollinators produced fewer fruits and had slow germination rates. In addition, the abundance of beeflies was positively correlated with seedling emergence (Table 4). Most pollinator variables showed significant spatial autocorrelation at different scales (Appendix B).

EFFECT OF POPULATION CHARACTERISTICS

Population variables also varied widely across populations. Population size ranged between 120 and 2050 plants and density of *E. popovii* between 0.01 and 0.56 individuals/m^2 (Table 1). *E. popovii* flower density (3.84±3.88 flowers/m^2, range: 0.33-18.6) was much lower than that of other, co-occurring plants (33.24±47.44 flowers/m^2, range: 6.1-228.4) (Table 1). Annual rainfall ranged between 493 and 737 mm (Table 1).

Only the density of co-occurring flowers showed a significant correlation with reproductive output. *E. popovii* plants growing in sites with high density of co-occurring flowers produced fewer fruits (Table 5). No significant effects on fecundity were found.

As with pollinator variables, most populational variables showed significant spatial autocorrelation at different scales (Appendix B), thus creating a complex geographic mosaic of biotic interactions and abiotic conditions.

	Seed weight			Germination time (T-50)			Fecundity		
	Coeff ± Std Error	t	*p* value	Coeff ± Std Error	t	*p* value	Coeff ± Std Error	t	*p* value
Pollinator variables									
Pollinator diversity (Hurlbert's PIE)	-1±0.96	-1.04	0.316	-1.15± 9.19	-0.125	0.902	9.162 ±14.42	0.635	0.538
Large-bee visitation rate (visits/hour)	-0.003±0.006	-0.42	0.677	0.039± 0.63	0.19	0.548	0.077 ±0.097	0.791	0.446
Small-bee visitation rate (visits/hour)	-0.001±0.011	-0.08	0.931	-0.117±0.11	-1.07	0.306	-0.095 ±-0.17	-0.557	0.589
Beefly visitation rate (visits/hour)	-0.002±0.004	-0.52	0.608	0.034±0.04	0.84	0.418	-0.002 ± 0.057	-0.042	0.967
Low-quality pollinator visitation rate (visits/hour)	0.006±0.007	0.84	0.414	**0.166±0.07**	**2.38**	**0.034**	0.105 ±0.195	0.333	0.745
Population variables									
Population size (reproductive individuals)	<0.001± <0.001	0.93	0.36	0.001±0.002	0.64	0.528	-0.002 ±0.004	-0.542	0.599
Population density (Nº of *E. popovii* /m^{2})	-0.038±0.37	-0.10	0.92	2.7±3.61	0.74	0.467	-5.862 ±5.34	-1.098	0.296
E. popovii flowers (flowers/population)	<0.001± <0.001	-0.66	0.519	<0.001±<0.001	-0.83	0.416	<0.001 ± <0.001	0.229	0.823
Co-occurring flowers density (flowers/m^{-2})	<0.001± <0.001	0.32	0.751	0.015±0.01	1.73	0.106	0.028 ± 0.082	0.344	0.737
Annual average rainfall (l m^{-2})	<0.001± <0.001	1.87	0.082	<0.001±<0.001	1.44	0.169	<0.001 ± <0.001	-0.017	0.987

TABLE 5: Coefficient, standard error, t and p values of spatially-explicit models relating variables related to seed quality and fecundity with pollinator and population variables separately.

DISCUSSION

Erysimum popovii coexists with the more abundant and more widely-distributed congeneric species *E. mediohispanicum*. *Erysimum mediohispanicum* blooms more profusely and produces many more ovules per individual than *E. popovii* (3535 and 839, respectively), and has a higher fruit set (0.82 ± 0.03 and 0.67 ± 0.14, respectively) (Gómez et al. 2010). The ratio seeds to ovules is similar in both species (0.70 ± 0.23 in *E. mediohispanicum* and 0.74 ± 0.16 in *E. popovii*) (Gómez 2005a), and seedling emergence rate is higher in *E. popovii* (0.14% seedlings per seed *versus* 0.09% in *E. mediohispanicum*) (Gómez 2005a). However, *E. popovii* seedling survival rate is lower in *E. popovii* than in *E. mediohispamicum* (0.06 and 0.21, respectively) (Gómez 2005a). As a result, the reproductive output of *E. popovii* (0.2% of the ovules turning into reproductive individuals) is considerably lower than that of *E. mediohispanicum* (1.1%). Thus, he low number of ovules per individual and the low seedling survival rate appear to be the two main factors limiting the reproductive success of the narrow endemic near threatened *E. popovii* (Cabezudo et al. 2005).

Many studies have demonstrated that seed weight is a good estimate of some reproductive variables, such as seed germination and seedling growth (Schaal 1980; Dolan 1984; Wulff 1986; Houssard & Escarre 1991; Navarro & Guitián 2003). However, seed weight showed no relationship with germination success, germination time or seedling emergence in *E. popovii*. Instead, we found a negative correlation between seed weight and seed set (proportion of ovules setting seeds). This pattern suggests a trade-off between seed size and seed number, as found in many other plant species (Jacquemyn et al. 2001; Guo et al. 2010).

Germination time in *E. popovii* was similar to that of *E. mediohispanicum*, with both species yielding T50 values of about 5 days. Similar results have been reported for other *Brassicaceae* species from the Iberian Peninsula (Maselli et al. 1999). In annual herbaceous communities, early germinating individuals may be better competitors, as short delays in emergence may be magnified into large differences in final biomass and fitness (Ross & Harper 1972; Rice 1990).

We found no relationship between germination success in the laboratory and seedling emergence in the field. These results highlight the importance of assessing germination under both situations. Laboratory germination tests provide estimates of seed performance under standard conditions, but not under suboptimal, often harsh, field conditions. Some studies have shown that populations exhibiting similar levels of germination success in the laboratory, respond very differently to a wide range of suboptimal environmental conditions (Summerfield 1972; Baskin and Baskin 2001).

Seven of our populations showed very low (<0.01) seedling survival, which may constitute a regeneration bottleneck. These populations were not particularly small, but could suffer drastic declines over the next generations if low levels of seedling survival persist. If so, these populations could experience pollen limitation as a consequence of pollinators depositing heterospecific pollen and/or inbreeding depression as a consequence of pollinators depositing self-pollen or pollen from closely related individuals (Herrera 1987; Pflugshaupt et al. 2002). The large differences among populations in recruitment, together with the absence of a dormant stage and the short life-cycle suggest a metapopulation structure for *E. popovii*. Given the small population sizes and the long distances between populations, together with the low dispersal ability found in other *Erysimum* species (Gómez 2007), local extinctions may become increasingly frequent and recolonization events increasingly rare.

We found no difference in reproductive outcome between regions. However, seed set, seed germination, seedling emergence and seedling survival showed spatial autocorrelation at different distances. This geographic pattern of variation in reproductive output suggests that variables affecting reproduction success are structured at different scales. The spatial variation between populations could be mediated by rainfall which usually presents high spatial correlation and germination responses have been commonly linked to particular characteristics of the precipitation regime, especially seasonal and annual variation (Clauss & Venable 2000; Venable 2007). Higher rates of seed germination would be expected to occur in regions with higher precipitation (Venable 2007). Rainfall has been shown to affect seedling emergence and survival in other Mediterranean plants (Espigares & Peco 1993, Rebollo et al. 2001). Nevertheless, we found no correlation between rainfall and reproductive outcome. A possible explanation for this discrepancy is that we used mean annual, instead of seasonal rainfall, which may be a stronger predictor of reproduction success (Crone & Lesica 2006). Summer drought is one of the main causes of seedling mortality in Mediterranean plants (Peñuelas et al. 2004; Harel et al. 2011). Thus, rainfall occurring in summer or shortly after seedling emergence may be a stronger determinant of *E. popovii* reproductive success than annual rainfall. Additional studies will be necessary to test this hypothesis.

Recruitment has been shown to be associated to abundance, diversity and identity of pollinators in many species (Gómez & Zamora 1999; Knight et al. 2005; Anderson & Johnson 2008; Gómez et al 2010). *Erysimum popovii* flowers are visited by a very diverse assemblage of pollinators (166 species), including large bees, small bees, beeflies, butterflies, hoverflies, beetles, and ants (Fernández et al. 2012, Fernández & Gómez 2012). We found a positive relationship between beefly visitation and *E. popovii* reproductive output via seedling emergence. This result may be a consequence of the high pollinating efficiency displayed by beeflies on *Erysimum*. Compared to other flower visitors, the foraging behavior of beeflies, with frequent movements between individual plants, maximizes the deposition of allogamous pollen per visit (Gómez et al. 2011; Fernández et al. 2012). On the other hand, visitation by low quality pollinators showed a negative relationship with fruit set and germination time. This result is remarkable, given the high diversity of pollinator species encountered, and is probably related to the negative relationship between *E. popovii* fruit set and density of co-occurring flowers. In fact, density of co-occurring flowers and visitation rates of low-quality pollinators are positively correlated ($r= 0.732$, $p<0.001$). Pollinator visitation rates in *E. popovii* are three times lower than those reported in other *Erysimum* species (Gómez et al. 2007; Fernández et al. 2012). Taken together, this evidence suggests that, in the presence of other pollen-nectar sources, high-quality pollinators tend to disregard *E. popovii* flowers. In addition, some low quality pollinators (ants) act as nectar thieves (Gómez et al. 2007), and others (small beetles) force their way into the flowers altering the appearance of the corolla. This could negatively affect visitation rates by high quality pollinators. In fact, correlation between low quality and beefly visitation was marginally non-significant.

In conclusion, *E. popovii* reproductive success varies widely among populations, and this variation seems to be mostly related to local pollinator assemblage and flower community. These differences do not show a regional structure. Rather, they generate a geographic mosaic, with some populations recruiting many new individuals every year and others showing no regeneration. This pattern suggests the occurrence of a metapopulational system as described in other species (Menges 1990; Bonet et al 2005; Rozenfeld et al 2008). Further studies exploring the connectivity between *E. popovii* populations and regions would help to evaluate the viability of this near threatened species across its entire distribution range.

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APPENDICES

Appendix A. *Spatial structure of reproductive outcomes, across the 21 Erysimum popovii populations studied.*

		Fruit set		Seed set		Germination Success		Seedling emergence		Seedling survival		Seed weight (mg/100seeds)		Germination time (T50)		Fecundity	
class	Distance	Moran's I	*p*	Moran's I	P	Moran's I	P	Moran's I	P	Moran`s I	P	Moran's I	P	Moran's I	P	Moran's I	P
1	1975	0.13	0.43	0.03	0.74	-0.06	0.69	0.08	0.70	-0.05	0.82	-0.01	0.94	-0.16	0.31	-0.07	0.75
2	14159	-0.15	0.25	0.05	0.62	-0.15	0.27	**-0.29**	**0.03**	**-0.36**	**0.03**	0.16	0.31	-0.004	0.99	-0.14	0.37
3	30893	0.13	0.31	-0.21	0.11	-0.02	0.91	0.01	0.95	-0.19	0.21	0.20	0.18	0.03	0.74	-0.13	0.39
4	43988	-0.23	0.07	-0.04	0.60	**-0.25**	**0.03**	0.09	0.44	-0.01	0.94	-0.22	0.18	-0.05	0.66	-0.01	0.96
5	53640	-0.21	0.11	0.02	0.86	0.02	0.91	0.07	0.51	0.02	0.93	-0.13	0.31	-0.01	0.39	-0.15	0.45
6	63051	-0.04	0.83	**-0.36**	**0.01**	0.15	0.29	-0.18	0.25	-0.16	0.33	-0.20	0.21	-0.02	0.78	0.13	0.45
7	75019	0.06	0.52	0.11	0.26	-0.01	0.95	0.004	0.94	0.005	1	-0.10	0.46	0.003	0.96	-0.03	0.84

APPENDIX B. *Spatial structure of pollinator and populational variables across the 21 Erysimum popovii populations studied.*

		Pollinator Diversity (Hurlbert's PIE)		Large Bees (visit/hour)		Small Bees (visit/hour)		Beeflies (visit/hour)		Low Quallity Pollinators (visit/hour)	
class	Distance	Moran's I	*p*	Moran's I	*p*	Moran's I	*p*	Moran's I	*p*	Moran's I	*p*
1	1975.491	0.244	0.09	0.009	0.96	-0.265	0.06	**0.402**	**0.00**	-0.05	0.764
2	14159.46	0.053	0.64	-0.035	0.76	-0.02	0.824	**-0.385**	**0.01**	**-0.29**	**0.045**
3	30893.78	**-0.388**	**0.05**	0.009	0.94	-0.022	0.824	-0.04	0.73	-0.046	0.704
4	43988.15	0.025	0.75	0.047	0.70	0.019	0.774	-0.036	0.81	0.074	0.603
5	53640.38	-0.098	0.39	0.028	0.81	0.034	0.714	0.073	0.55	-0.072	0.538
6	63051.26	**0.341**	**0.05**	-0.14	0.33	-0.076	0.578	0.237	0.13	-0.034	0.849
7	75019.24	**-0.718**	**0.00**	**-0.51**	**0.00**	-0.077	0.377	**-0.564**	**0.01**	0.045	0.623

		Population Size (Nº of *E. popovii* /population)		Population density (Nº of *E. popovii* /m^2)		*E. popovii* flowers (flowers/population)		Density of co-occurring flowers (flowers/m^2)		Rainfall (l/m^2)	
class	Distance	Moran's I	*p*	Moran's I	*p*	Moran's I	*p*	Moran's I	*p*	Moran's I	*p*
1	1975.491	**0.322**	**0.02**	0.011	0.92	-0.01	0.96	0.117	0.32	**0.729**	**0.005**
2	14159.46	0.105	0.44	-0.261	0.08	0.115	0.362	-0.143	0.17	0.052	0.714
3	30893.78	**-0.437**	**0.01**	0.006	0.96	0.028	0.834	-0.107	0.28	**-0.441**	**0.015**
4	43988.15	-0.303	0.06	-0.131	0.26	-0.209	0.106	0.035	0.70	0.108	0.387
5	53640.38	0.118	0.36	0.09	0.38	-0.011	0.95	**-0.388**	**0.00**	**0.373**	**0.05**
6	63051.26	**-0.359**	**0.01**	0.089	0.50	**-0.312**	**0.05**	0.027	0.82	**-0.601**	**0.005**
7	75019.24	0.135	0.27	-0.13	0.22	-0.022	0.769	0.106	0.36	**0.729**	**0.005**

APPENDIX C. *Pearson's Correlation Coefficient (r) between several pollinator variables.*

	Beefly visitation rate (visits/hour)	Large bee visitation rate (visits/hour)	Small bee visitation rate (visits/hour)	Low Quality pollinator visitation rate (visits/hour)
Pollinators diversity (Hurlbert's PIE)	r=-0.350 p=0.12	**r=-0.80** **p<0.001**	r=0.16 p=0.47	r=0.17 p=0.45
Beefly visitation rate (visits/hour)	-	r=0.34 p=0.13	r=-0.06 p=0.81	r=-0.40 p=0.07
Large bee visitation rate (visits/hour)		-	r=0.12 p=0.59	r=-0.16 p=0.65
Small bee visitation rate (visits/hour)			-	r=0.11 p=0.65
Low quality pollinator visitation rate (visits/hour)				-

APPENDIX D. *Pearson's Correlation Coefficient (r) between populational variables.*

Population Size (Nº of *E. popovii* /population)	Population density (Nº of *E. popovii* /m^2)	*E. popovii* flowers (flowers/population)	Density of co-occurring flowers (flowers/m^2)	Rainfall (l/m^2)
Population Size (Nº of *E. popovii* /population)	r=0.262 p=0.250	**r=0.852** **p<0.001**	r=0.954 p=0.681	r=-0.214 p=0.352
Population density (Nº of *E. popovii* /m^2)	-	r=0.166 p=0.471	r=-0.003 p=0.990	r=-0.036 p=0.877
E. popovii flowers (flowers/population)		-	r=0.025 p=0.913	r=0.002 p=0.991
Density of co-occurring flowers (flowers/m^2)			-	r=-0.013 p=0.955
Rainfall (l/m^2)				-

APPENDIX E. *Pearson's Correlation Coefficient (r) between reproductive outcome variables.*

	Seed set	Seed weight (mg/100seeds)	Germination success	Germiantion time (T50)	Seedling emergence	Seedling production
Fruit set	r=0.118 p=0.642	r=0.087 p=0.733	r=0.053 p=0.836	**r=-0.663** **p=0.003**	r=0.368 p=0.133	r=0.327 p=0.186
Seed set	-	**r=-0.513** **p=0.029**	r=-0.445 p=0.064	r=-0.134 p=0.596	r=0.061 p=0.809	r=0.027 p=0.915
Seed weight (mg/100seeds)		-	r=0.428 p=0.077	r=0.209 p=0.404	r=-0.395 p=0.105	r=-0.291 p=0.242
Germination			-	r=-0.040 p=0.874	r=0.171 p=0.498	r=0.054 p=0.831
Germiantion time (T50)				-	**r=-0.470** **p=0.049**	r=-0.225 p=0.369
Seedling emergence					-	**r=0.566** **p=0.014**
Seedling production						-

APPENDIX F. *Mean of number of flower, number of fruit, number of ovules, number ovules in fruit, number of fertilized ovules, number of seed, number of seedling and number of juveniles produced per each Erysimum popovii plant.*

Population	Flowers	Fruits	Ovules	Ovules in fruit	Fertilized ovules	Seeds	Seedlings	Juveniles
Ep01	29.4	18.20	1657.26	542.00	444.12	423.36	239.67	0.00
Ep03	26.8	19.01	912.03	735.30	598.03	588.42	151.18	1.69
Ep04	21.27	16.33	386.09	353.87	295.59	293.90	60.24	9.38
Ep05	39.27	28.13	648.38	460.77	363.67	360.80	39.58	0.27
Ep07	29.87	21.82	363.65	265.52	173.17	165.96	5.31	0.23
Ep08	21.8	15.02	475.70	286.95	239.11	233.99	13.66	0.71
Ep09	37.23	27.53	512.79	548.35	454.96	442.84	12.73	2.22
Ep10	79.50	67.37	1272.46	437.13	306.09	299.07	48.03	1.33
Ep14	77.80	58.94	1923.10	1288.80	904.86	892.05	240.14	1.78
Ep15	69.27	54.97	1550.43	1165.36	966.81	954.50	130.68	1.24
Ep16	52.00	38.70	1104.91	808.83	572.58	564.03	152.60	6.39
Ep19	31.33	23.09	830.27	689.07	494.18	482.80	36.22	0.13
Ep20	34.41	24.43	617.80	457.82	328.99	309.49	66.55	2.49
Ep21	37.17	27.77	809.41	582.06	460.90	446.05	43.72	1.02
Ep23	41.37	27.41	723.44	593.48	478.40	466.24	46.86	0.24
Ep27	19.33	13.33	529.92	386.70	299.45	282.50	60.60	0.13
Ep28	32.87	22.53	592.62	476.28	366.75	365.17	26.14	0.46
Ep30	15.23	10.77	198.95	140.66	114.58	111.96	19.94	0.60
Total	37.58±17.89	27.36±15.25	839.40±482.02	567.72±293.05	436.79±222.36	426.84±220.46	77.44±73.91	1.68±2.43

APPENDIX G*. Abundance and diversity of pollinators visiting Erysimum popovii in the 21 study populations.*

Population	Pollinator species	Pollinator diversity (Hurbert's PIE)	Large Bees (visit/hour)	Small Bees (visit/hour)	Beeflies (visit/hour)	Low Quallity Pollinators (visit/hour)
Ep01	21	0.83	12.15	2.55	0.45	11.10
Ep02	22	0.94	1.64	10.50	2.59	9.27
Ep03	13	0.73	33.47	5.05	44.84	3.16
Ep04	24	0.82	19.55	4.84	19.74	7.94
Ep05	22	0.88	3.39	2.26	3.74	6.09
Ep07	13	0.78	4.04	2.09	11.87	5.22
Ep08	20	0.91	2.63	5.81	2.25	8.81
Ep09	13	0.62	44.75	8.50	6.75	5.00
Ep10	23	0.86	9.33	3.85	12.30	7.26
Ep13	16	0.85	4.68	6.10	0.00	32.34
Ep14	32	0.94	11.33	21.67	11.33	8.83
Ep15	28	0.94	3.00	7.88	4.50	9.25
Ep16	18	0.90	0.91	1.96	2.94	8.53
Ep19	29	0.92	7.00	4.78	7.22	4.56
Ep20	25	0.84	0.82	3.55	0.36	11.36
Ep21	15	0.67	39.33	7.33	12.00	8.00
Ep22	16	0.85	3.67	8.17	14.67	4.50
Ep23	20	0.87	12.40	3.87	16.53	3.73
Ep27	11	0.79	9.60	3.12	48.24	2.64
Ep28	21	0.79	1.15	8.31	14.42	3.58
Ep30	14	0.84	14.40	9.00	17.40	19.00
Total	166±5.80	0.95±0.08	12.74±13.38	5.91±4.57	13.16±13.51	7.45±3.93

CAPÍTULO 2

POLLEN LIMITATION IN A NARROW ENDEMIC PLANT: GEOGRAPHICAL VARIATION AND DRIVING FACTORS

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Pollen limitation in a narrow endemic plant: geographical variation and driving factors.

ABSTRACT

Pollen limitation may have important consequences for the reproduction and abundance of plant species. It may be especially harmful to endangered and endemic plants with small populations. In this study we quantify the effect of pollen limitation on seed production and seedling emergence in an endangered narrow endemic crucifer, *Erysimum popovii*. We conducted a pollen addition experiment across the entire geographic distribution of the species, and explored the effect of pollinator assemblage, plant population size and density, and other habitat variables on pollen limitation intensity in 13 populations. We supplemented flowers in 20 plants per population with allogamous pollen. To account for potential resource reallocation, we used two types of control untreated flowers: internal control flowers from the same individual as the supplemented flowers, and external control flowers from other individuals. Our results indicate that *E. popovii* is pollen-limited in most of the populations studied, but only through seed production, since pollen supplementation did not enhance seedling emergence. Beefly abundance was associated with among-population differences in pollen limitation intensity. Populations in which beeflies were more abundant were less pollen-limited. In contrast, the abundance of other flower visitors, such as large bees or butterflies, was not associated with pollen limitation. Annual rainfall and bare soil cover were associated with the intensity of pollen limitation across populations.

KEYWORDS: *Beefly · Pollination · Plant reproductive ecology · Erysimum · Endangered plant*

INTRODUCTION

Plant reproduction may be limited by the availability of resources (Suzuki 2000; Griffin and Barrett 2002), inadequate pollen receipt (Ashman et al. 2004; Knight et al. 2005; Aizen & Harder 2007), or a combination of both factors (Haig and Westoby 1988). Pollen limitation appears to be common across Angiosperms (Ashman et al. 2004; Knight et al. 2005). According to recent reviews, between 62 and 73 % of all insect-pollinated plants show evidence of inadequate pollen receipt (Ashman et al. 2004; García-Camacho & Totland 2009).

Pollen limitation may be caused by a decrease in pollen quantity or quality (Aizen & Harder 2007; Harder and Aizen 2010). Pollen quantity limitation occurs when pollinators are scarce or ineffective (depositing low amounts of pollen grains per visit) (Johnston 1991; Gómez et al. 2010). Pollen quality limitation occurs when pollinators deposit incompatible pollen, self-pollen or pollen from closely related individuals, which may produce inbreeding depression and seed quality reduction (Herrera 1987; Pflugshaupt et al. 2002). Consequently, the potential effects of pollen limitation on reproductive success should ideally be measured in each of the plant´s life-cycle reproductive stages (Ehrlén & Eriksson 1995). Yet, as noted by Knight et al. (2005), most studies only examine the consequences of pollen limitation on fruit and seed set, and few studies measure potential effects on post-dispersal stages (Ehrlén & Eriksson 1995; Price et al. 2008; Gómez et al. 2010).

The consequences of pollen limitation may be dramatic for plants. Reproductive failure due to pollen limitation may lessen population growth and long-term viability, resulting in declines in population size (Eriksson & Jakobsson 1998; Knight et al. 2005). Thus, pollen limitation may strongly influence the distribution and abundance of plant species and populations (Eriksson & Jakobsson 1998). Severe and consistent pollen-limitation may even trigger local extinctions in fragmented or heterogeneous habitats harboring small populations (Aguirre & Dirzo 2008; Hill et al. 2008). Reproductive failure due to pollen limitation is likely to be stronger in small, isolated populations than in large, well-connected populations (Aizen & Feinsinger 1994). This is especially important for the persistence of endemic plants, which have restricted distribution areas, and usually show lower fertility than their widespread congeners (Lavergne et al. 2004). Exploring the importance of pollen limitation for the reproduction of narrow endemic plants living in fragmented habitats is thus essential to understand and predict the consequences of the anthropogenic-mediated pollinator crisis on the conservation of plant populations (Allen-Wardell et al. 1998; Vamosi et al. 2006; Eckert et al. 2010).

In animal-pollinated plants, insufficient pollen deposition or deposition of low-quality pollen is mostly caused by pollinator assemblage characteristics, such as pollinator abundance, diversity and identity. Many studies have found pollen limitation to be associated with decreased pollinator abundance (Baker et al. 2000; Cunningham 2000; Cosacov et al. 2008; González-Varo et al. 2009; Gómez et al. 2010). In addition, pollen limitation may be related to decreased pollinator diversity (Gómez et al. 2010). This relationship appears when the plant’s probability of being visited by effective pollen vectors increases with pollinator diversity (Perfectti et al. 2009). Pollen limitation is also related to the identity of pollinators, as different flower visitors differ in pollinating effectiveness and may have contrasting effects on plant fitness (Klein et al. 2003; Gómez et al. 2007; Ne’eman et al. 2010). In addition to pollinator assemblage traits, some characteristics of the plant population itself may influence pollen limitation intensity directly or indirectly. One example of direct effects is strong fragmentation, which may result in genetic impoverishment, thus setting a limit to the quality of the pollen deposited, irrespective of pollinator community (Byers 1995). Indirect effects occur when plant population characteristics modify pollinator composition and behavior. For example, plant population size, habitat fragmentation, co-occurring flower composition and altitude influence the community and activity of flower-visiting insects, and therefore the intensity of pollen limitation (Totland 2001; González-Varo et al. 2009; Jakobsson et al. 2009).

E. popovii populations	Region	Coordinates (UTM)	Elevation (m a.s.l.)	Population Size (Nº *E. popovii* plants)	Density (*E. popovii* plants/m^2)	Flowers per plant[1]	Habitat	Mean Annual Temp. (ºC)	Accumulated Annual Precipitation (mm)
01	Mencal, Granada, Spain	30S 0487836 4155063	982	600	0.015	29.4±19.08	Scrub	13.9	493.5
02	S. Arana, Granada, Spain	30S 0451977 4128858	1255	340	0.011	29.78±12.61	Grassland+Rocks	12.9	579.6
03	S. Nevada, Granada, Spain	30S 0456751 4109531	1596	280	0.037	26.8±16.16	Scrub	10.7	510.2
04	La Peza, Granada, Spain	30S 0467701 4118300	1503	300	0.073	21.27±9.13	Grassland+Rocks	11.8	577.0
05	S. Jabalcuz, Jaen, Spain	30S 0425545 4177799	1321	420	0.197	39.27±34.34	Scree	12.1	725.6
07	S Mágina, Jaen, Spain	30S 0453888 4176684	2000	500	0.135	29.87±28.42	Scrub	9.2	549.6
08	S. Jabalcuz, Jaen, Spain	30S 0425519 4177931	1324	400	0.173	21.8±12.51	Pine Forest	12.5	726.7
09	La Peza, Granada, Spain	30S 0472834 4119460	1693	130	0.085	37.23±35.84	Grassland	10.4	609.1
10	S. Jabalcuz, Jaen, Spain	30S 0424122 4177754	1060	450	0.064	79.5±77.45	Scree	13.5	737.3
13	S. Arana, Granada, Spain	30S 0454337 4131901	1738	370	0.087	29.97±25.11	Grassland+Rocks	9.9	642.4
14	S. Jabalcuz, Jaen, Spain	30S 0426894 4180210	970	120	0.025	77.8±116.95	Grassland+Rocks	14.2	689.0
15	S. Jabalcuz, Jaen, Spain	30S 0425148 4179387	1080	650	0.062	69.27±48.81	Scrub	13.9	648.0
16	S. Jabalcuz, Jaen, Spain	30S 0425152 4179487	1060	150	0.038	52±54.09	Pine Forest	13.8	640.5
19	Mencal, Granada, Spain	30S 0483826 4151106	1188	420	0.246	31.33±27.37	Grassland	12.4	496.7
20	S. Arana, Granada, Spain	30S 0451048 4130531	1270	350	0.069	34.4±21.86	Scrub	12.6	640.6
21	S. Arana, Granada, Spain	30S 0452839 4131133	1498	280	0.113	37.17±20.63	Grassland	11.1	675.5
22	S. Arana, Granada, Spain	30S 0453676 4128236	1380	620	0.069	33.57±19.36	Grassland	11.5	569.7
23	La Peza, Granada, Spain	30S 0472949 4119278	1720	750	0.556	41.37±25.75	Grassland	10.3	602.6
27	S. Nevada, Granada, Spain	30S 0457430 4109448	1486	340	0.176	19.33±11.50	Grassland	11.2	540.6
28	S Mágina, Jaen, Spain	30S 0455789 4176686	1661	1650	0.193	32.87±21.29	Grassland	10.9	559.4
30	S Mágina, Jaen, Spain	30S 0453436 4176812	1898	2050	0.133	15.23±6.388	Grassland	9.4	582.7

Table 1. *Location and characteristics of the 21 E. popovii populations studied. [1] Mean ± SD*

The general goal of this study is to describe geographical variation of pollen limitation on seed and seedling production in an endangered Mediterranean endemic herb, *Erysimum popovii*, and to explore the potential factors influencing pollen limitation. Our specific objectives are: (1) to determine the geographic variation in pollen limitation throughout the entire distribution area of the species; (2) to establish at what stage, pre-dispersal (seed production) or post-dispersal (emergence rate), is pollen limitation more intense; (3) to study the association between flower visitor community and pollen limitation; and (4) to study the association between population characteristics (size, density of co-flowering species, habitat composition) and pollen limitation.

MATERIALS AND METHODS

STUDY SYSTEM

Erysimum popovii Rothm. (Brassicaceae) is a narrow endemic plant from Southeastern Spain, categorized as "Near Threatened" in the Red List of Andalusia Vascular Flora (Cabezudo et al. 2005). The species inhabits rocky areas and shrubland gaps in montane areas from 900 to 2000 m. a.s.l. It is a biennial to perennial monocarpic herb, usually producing tens of flowers (mean±SD = 37.6±17.9 flowers; Table 1) on a variable number of reproductive stalks. Flowers are hermaphroditic and slightly protandrous, with four bright purple petals (Blanca et al. 1992; Blanca et al. 2009). Flower lifespan is 1-2 days, depending on environmental and pollination conditions (authors' personal observation). Like other *Erysimum* species (Gómez 2005), *E. popovii* is self-compatible, but requires pollen vectors for full seed set (plants from which pollinators are excluded produce seeds, but in low numbers, authors' personal observation).

The study was conducted in 21 *Erysimum popovii* populations located in the Baetic Mountain range (Granada and Jaen provinces, southern Spain, Table 1), spanning the entire geographic range of the species (Fig. 1). Maximum distance between populations was 80 km. The area has a characteristic Mediterranean climate with cool wet winters and warm dry summers.

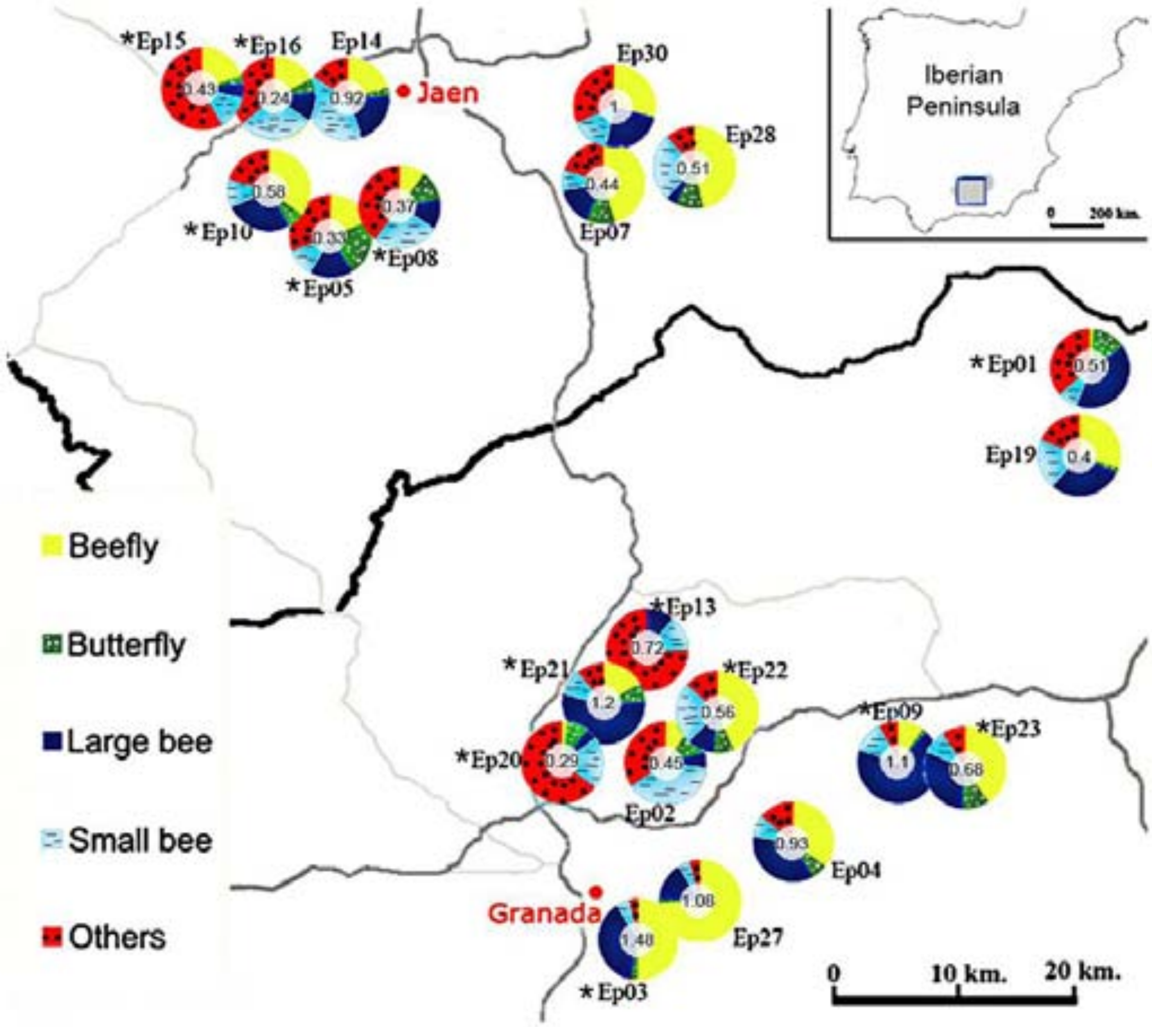

Figure 1. *Map showing locations of the 21 Erysimum popovii populations studied, and composition of flower visitor functional groups at each site. Numbers are flower visitation rates (in visits min*$^{-1}$*); asterisks denote populations in which we could analyze pollen limitation.*

POLLEN ADDITION EXPERIMENT

We conducted a pollen-supplementation experiment in the spring of 2009. In each of the 21 populations, we labeled 50 plants of average size during peak bloom. In 20 randomly designated plants, we administered pollen to four flowers from the central part of the flowering stalk (PA treatment). Pollen was collected in the early morning from newly opened flowers of donor individuals located at least 10 meters from the receiving individual. In each receiving individual we selected four additional flowers that were left untreated and served as internal controls (IC treatment). IC flowers were located immediately above or below PA flowers. By selecting PA and IC flowers from the central part of the flowering stalks we expected to avoid potential confounding effects of flower position on reproductive outcome (Wesselingh 2007). In the 30 remaining plants, we selected 3 flowers as external controls (EC treatment). EC flowers were used to detect possible effects of pollen supplementation on resource allocation (Wesselingh 2007). Domestic and wild ungulates consumed some of our experimental plants. As a result, we could recover enough individuals to perform statistical analyses in only 13 of the 21 populations. For this reason, all subsequent pollen limitation analyses are based on 13 populations.

We measured the effect of pollen limitation during both the pre-dispersal stage of the plant's reproductive success (seed production), and the post-dispersal stage (seedling emergence rate). Seed production was estimated as seeds per flower. At the end of the fruiting season, we collected ripe fruits produced by PA, IC and EC flowers. For each fruit, ovules, aborted seeds and ripe seeds were counted in the laboratory using a magnifying glass. We were able to discern ripe seeds, aborted seeds and unfertilized ovules because, as in other crucifers, ripe seeds are light brown and bigger, and aborted seeds are dark brown, with shriveled cotyledons and embryo (Gómez et al. 2010). By contrast, unfertilized ovules are creamy white and smaller. Seedling emergence rate was measured as the proportion of seeds producing seedlings. Due to the relatively low number of seeds obtained in some mother*treatment combinations, we decided to pool all seeds within each treatment and population, and randomly selected 24 seeds per treatment and population (PA, IC and EC). In February 2010 these seeds were sown in seedbeds in a greenhouse, and emergence was monitored weekly until April.

To determine the magnitude of pollen limitation, we used a pollen limitation (PL) index calculated as 1-(RS_C/RS_{PA}), where RS_C is the mean reproductive success of the control treatment and RS_{PA} the mean reproductive success of the pollen addition treatment. This index has a straightforward biological interpretation (Lázaro and Traveset 2006). Positive values result from higher reproductive success in PA than C, thus indicating pollen limitation, while zero or negative values indicate absence of pollen limitation. We calculated the PL index for both components of reproductive success (seed production and seedling emergence), and for the comparisons PA *vs* IC and PA *vs* EC (henceforth PL_{IC} and PL_{EC}). The difference between these two PL estimates provides a measure of resource allocation as a consequence of the experimental supplementation of pollen (Gómez et al. 2010).

FLOWER VISITOR ASSEMBLAGE

We conducted surveys of floral visitors in each of the 21 populations during the springs of 2008 and 2009. Each population was sampled on several days throughout its flowering period. Groups of plants with approximately 100 flowers were tagged and repeatedly observed over the course of the day (from 11:00 to 17:00) and all insects seen actively visiting the flowers (collecting pollen and/or nectar) were noted. We tried to record a minimum of 200 flower-pollinator contacts per population because this number provided a good estimate of overall pollinator assemblage in *E. mediohispanicum* (Gómez et al. 2007). Total survey time per population ranged from 190 to 795 minutes. We observed a total of 5169 flower visits (193 to 331 per population). Specimens (n=486) of most morphospecies were captured for later identification in the laboratory.

We estimated flower visitation rate as the number of flower visits observed per minute of observation. We also quantified the visitation rate of each type of flower visitor, considering the following five functional groups: Large bees (10 mm or more in body length), small bees (less than 10 mm in body length), butterflies (mostly Rhopalocera, all nectar collectors), beeflies (long-tongued Bombyliidae) and others (including ants, beetles, hoverflies and other minor groups previously shown to have low pollinating efficiency on *Erysimum*; Gómez et al. 2007, 2008, 2009). Finally, we estimated the overall diversity of the flower visitor community in each population using Hurlbert's PIE (EcoSim 7, Gotelli & Entsminger 2009). This index ranges between 0 (low diversity) and 1 (maximum diversity), and measures the probability that two randomly sampled individuals from the community belong to different species.

PLANT POPULATION CHARACTERISTICS

We measured the following variables to characterize the *E. popovii* populations: Population size (all reproductive individuals in each population), altitude (meters above sea level measured with a GPS), annual rainfall (in mm, obtained from the Digital Climatic Atlas of the Iberian Peninsula; Ninyerola et al. 2005), density of co-occurring flowering plants (flowers per m^2), woody plant cover (percent area occupied by woody plants), bare soil cover (percent area occupied by bare soil) and rock cover (percent area occupied by rocks). Flowering plant density was measured along three transects of 10 x 2 meters in which we counted the number of flowers of species blooming at the same time as *E. popovii*. Habitat cover was estimated along three additional transects of 25 x 2 meters in which, every 0.5 meters, we scored microhabitat type in the center and at the two edges of the transect width.

DATA ANALYSIS

Significance of PL index for seed production of each population was obtained with bootstrapping, running 1000 replicates per population (Boot package, R Development Core Team 2008). Bootstrapping could not be applied to post-dispersal PL results because, by pooling all seeds within each treatment and population, internal variation required for this analysis was lost (Davison and Hinkley 1997). Thus, we used contingency tables to compare seedling emergence rates across treatments within each population.

Spatial autocorrelation of PL indices was analyzed with Moran's I coefficient at different distance classes (Rangel et al. 2010). The number of distance classes was established by the default function of the software (SAM v. 4.0; Rangel et al. 2010). The relationship of flower-visitor community and population characteristics with pollen limitation intensity was explored by means of a model selection approach. We run a set of models including all possible combinations of independent variables. Since pollen limitation was spatially autocorrelated (Appendix A), these models were spatially-explicit, including X and Y geographic coordinates as co-variables to control for the possible effects of spatial distribution. Prior to running these models, we checked for multicollinearity by performing pairwise Pearson's correlations amongst variables. Since no strong correlation appeared between variables (Appendices B and B), we included all independent variables in the models. Due to the high number of independent variables with respect to the number of samples (13 populations), we run separate models for flower-visitor and population variables. To further avoid potential problems derived from the small number of populations, we decided not to include more than three independent variables in each model. We thus run all models resulting from all possible combinations of one, two or three variables. All analyses were performed with SAM v. 4.0 (Rangel et al. 2010). Because the two PL indices were consistent (see below), we only show outcomes of PL_{EC}, which is more robust to resource reallocation (Wesselingh 2007).

To select the best model(s), we used an information-theoretic approach (Burnham and Anderson 2002; Richard 2005; Stephens et al. 2007). We first selected those models providing an appropriate goodness of fit ($P < 0.05$; Grace 2006). For this subset of candidate models, we calculated the Akaike information criterion (AIC) and the Akaike weights (AIC_{wi}). All models having $AIC_{wi} > 0.2$

were considered an appropriate representation of the raw data (Burnham and Anderson 2002). By means of a multimodel inference process, we determined the importance of each independent variable across all models weighted by their AIC values (Richard 2005).

RESULTS

POLLEN LIMITATION

Despite large differences among populations, flowers from PA treatments produced more seeds than the two control treatments in all populations but one (Fig. 2). Excluding population 3 (in which IC flowers produced more seeds than PA flowers), PA flowers produced a mean of 3.76 more seeds (SD = 2.03; range = 0.74 - 7.37; n = 12 populations) than IC flowers. When considering entire plants, this represents a mean increase of 155 ± 115 seeds per individual (range = 26 - 416). Of the 13 populations analyzed, PL_{IC} was significant in eight, and PL_{EC} in ten (Table 2), although PL values were mostly moderate (PL_{IC}=0.18-0.45; PL_{EC}= 0.16-0.49). When pooling all populations, pollen limitation for seed production was significantly different from zero for both PL indices (Table 2).

On the other hand, we found no significant pollen limitation for seedling emergence rate ($PL_{IC} = 0.012$, $p=0.46$; $PL_{EC} = -0.039$, $p=0.31$). In fact, only two populations (Ep01 and Ep15) were pollen limited for seedling emergence and only when comparing PA and EC treatments (Table 2).

We did not find a consistently better performance of EC treatment compared to IC treatment (binomial test of EC performance > IC performance, P = 0.5), with no differences between EC and IC in seed production when data from all populations were lumped together (Fig. 2). In addition, in most populations (9 out of 13), PL_{IC} and PL_{EC} values were similar (Table 2). These results provide no evidence of reallocation between internal control and pollen-supplemented flowers.

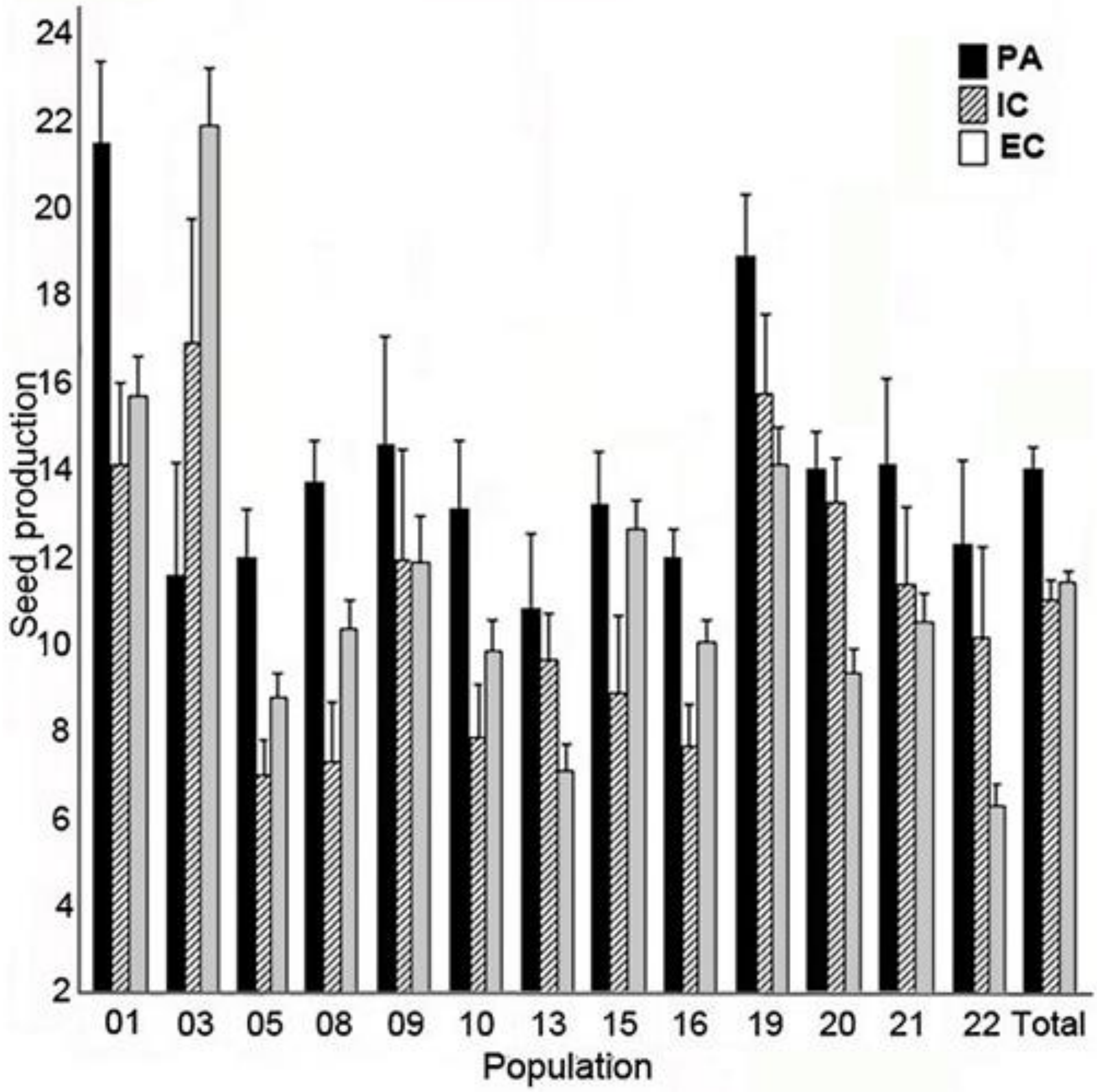

Figure 2. *Seed production (seeds per flower) in PA (Pollen Addition), IC (Internal Control) and EC (External Control) flowers from populations for which we could conduct bootstrapping analysis of pollen limitation (see Table 2). Bars are means (+SE).*

E. popovii Population	N. of individuals treated	N. of individuals recovered	Seed production		Seedling emergence rate	
			PL_{IC}	PL_{EC}	PL_{IC}	PL_{EC}
01	20	8	0.30 (0.19, 0.5)	0.27 (0.11, 0.45)	0.33 (0.08)	0.40 (0.05)
02	20	3	0.4	0.016	0.15 (0.17)	0.04 (0.38)
03	20	11	-0.89 (-0.89, 0.03)	-0.92 (-1.84, 0.27)	-0.17 (0.39)	-0.50 (0.07)
04	20	5	0.28	0.19	-0.02 (0.71)	0.29 (0.06)
05	20	14	0.36 (0.29, 0.54)	0.27 (0.12, 0.43)	-1 (0.11)	0.60 (0.16)
07	20	2	-0.25	0.14	0 (0.99)	-1 (0.28)
08	20	7	0.45 (0.27, 0.67)	0.24 (0.11, 0.38)	-	0 (0.99)
09	20	6	0.14 (-0.07, 0.45)	0.18 (-0.07, 0.45)	0 (0.99)	- 1 (0.01)
10	20	7	0.39 (0.3, 0.5)	0.25 (0.29, 0.5)	0 (0.05)	-8 (0.005)
13	20	16	-0.15 (-0.02, 0.25)	0.34 (0.08, 0.65)	-	-
14	20	5	0.12	-0.41	0.18 (0.1)	0.04 (0.16)
15	20	11	0.25 (0.05, 0.62)	0.043 (-0.17, 0.27)	0.20 (0.19)	0.60 (0.04)
16	20	12	0.35 (0.21, 0.5)	0.16 (0.03, 0.31)	-0.62 (0.12)	-0.87 (0.06)
19	20	17	0.18 (0.04, 0.29)	0.25 (0.1, 0.4)	0 (0.25)	-1 (0.04)
20	20	16	0.016 (-0.09, 0.19)	0.33 (0.24, 0.43)	-0.05 (0.5)	-0.05 (0.5)
21	20	14	0.22 (0.07, 0.31)	0.26 (0.05, 0.48)	0 (0.33)	0.10 (0.23)
22	20	8	0.17 (-0.01, 0.36)	0.49 (0.34, 0.67)	-1.17 (0.04)	0 (0.26)
23	20	3	0.36	0.21	-	0.50 (0.11)
27	20	5	0.26	0.24	0 (0.28)	0.11 (0.21)
28	20	5	0.12	0.01	0.75 (0.15)	-2 (0.01)
30	20	0	-	-	-	-
Total	420	175	0.21 (0.16, 0.27)	0.19 (0.12, 0.25)	0.012 (0.46)	-0.039 (0.31)

Table 2. *Pollen limitation (PL) intensity in each population. The significance of PL for seed production was determined through bootstrapping with 1000 permutations (only in those populations in which more than 8 individuals could be recovered). Populations in which the bootstrapping 95% confidence interval (in parentheses) does not include zero have significant pollen limitation. For seedling emergence rate, the significance of PL was tested with Fisher's exact test (p-values in parentheses). "Total" is the number of individuals from all populations"*

FLOWER VISITORS

The flower visitor community of *Erysimum popovii* was very diverse, including large bees, small bees, beeflies, butterflies, hoverflies, beetles and ants (Fig. 1). Taking together all populations, we observed 166 insect species visiting the flowers of *E. popovii*, ranging from 11 to 32 species per population (Appendix D). Flower visitor diversity ranged between 0.62 and 0.94 (Hurlbert's PIE index). Mean visitation rate was 0.348 visits per flower per hour (range=0.084-1.050) (Appendix D). Large bees and beeflies were the most abundant flower visitors (Fig. 1).

FACTORS ASSOCIATED WITH POLLEN LIMITATION

As we found no evidence of pollen limitation on germination rates, analyses exploring the factors associated to pollen limitation were conducted only for seed production. The visitation rate of beeflies was the main variable influencing pollen limitation, with an importance value of 0.966, which is much higher than importance values of the others variables (Table 3). Consequently, the best model included only beefly visitation rate (Model A; Tables 3 and 4). According to this model, beefly visitation rate was negatively correlated with the intensity of pollen limitation (Table 3), so that populations with higher beefly abundance showed less pollen limitation. The abundance of the other flower visitor groups (large bees, small bees, butterflies and others), as well as the visitation rate and diversity of the whole flower visitor assemblage revealed no relationship to pollen limitation (Table 3). These variables showed very low importance values (Table 3), and did not appear in the selected models (Table 4).

When exploring the effect of population characteristics, we found that annual rainfall and bare soil cover were the two most important variables (0.50 and 0.44 importance values, respectively; Table 3). The selection model process yielded two equally good models (Models B and C, Table 4). Annual rainfall was positively and significantly related to pollen limitation intensity (Table 3). That is, pollen limitation was stronger in populations receiving more precipitation. Model C only included bare soil cover, which was marginally and negatively correlated with PL_{EC} (Table 3). In other words, pollen limitation tended to be lower in populations with extensive bare soil cover. The remaining population characteristics did not correlate with pollen limitation intensity (Table 3).

Multi-model inference		Statistical outcomes of best models			
	Importance	Coeff ± Std Error	t	P Value	Model
Flower visitor variables					
Beefly visitation rate	0.966	-1.604 ± 0.369	-4.347	0.002	A
Small bee visitation rate	0.046				
Large bee visitation rate	0.042				
Flower visitor diversity (Hurlbert's	0.041				
Flower visitation rate (insects min^{-1})	0.039				
Other pollinators visitation rate	0.039				
Butterfly visitation rate	0.037				
Population variables					
Annual rainfall (mm)	0.504	0.010 ± 0.004	2.34	0.047	B*
Bare soil cover (%)	0.44	-5.56 ± 2.43	-2.28	0.052	C
Rock cover (%)	0.108				
Co-occurring flower density (plants	0.09				
Population size (Nº of *E. popovii*	0.077				
Woody plant cover (%)	0.044				
Altitude (m a.s.l.)	0.042				

Table 3. *Variables influencing pollen limitation intensity for seed production, based on 13 populations in which bootstrapping analysis could be conducted. For each variable, the importance value obtained from a multi-model inference using AIC weights averaged across 127 models. Mean±SE coefficients were obtained from the model-averaging process are shown. The statistical outcomes of the best models are given in Table 4. *To obtain meaningful slopes in Model B, rainfall was measured in m instead of mm.*

Model	Variables	AIC_{wi}	Model	Variables	AIC_{wi}
Flower visitor variables			Population variables		
Model A	3	0.797	Model B	10	0.312
Model 2	3, 7	0.036	Model C	13	0.277
Model 3	3, 4	0.035	Model 3	10,13	0.075
Model 4	3, 6	0.028	Model 4	14	0.046
Model 5	1, 3	0.025	Model 5	11	0.041
Model 6	3, 5	0.022	Model 6	10,14	0.037
Model 7	2, 3	0.02	Model 7	11,13	0.024

Table 4. *Outcome of the model selection process for flower visitor and population variables. Only models having an AIC weight (AIC_{wi}) ≥ 0.02 are shown. VARIABLES: 1.- Flower visitation rate. 2.- Flower visitor diversity. 3.- Beefly visitation rate. 4.- Butterfly visitation rate. 5.- Large bee visitation rate. 6.- Small bee visitation rate. 7.- Others visitation rate. 8.- Population size. 9.- Altitude. 10.- Annual rainfall. 11.- Co-occurring flowering plants. 12.- Woody plant cover (%). 13.- Bare soil cover (%). 14.- Rock cover (%).*

DISCUSSION

Our study has shown that *E. popovii* reproduction was pollen limited throughout most of its distribution range. This finding was somewhat unexpected because *E. popovii* is self-compatible and its flowers are visited by over 166 insect species belonging to very disparate functional and taxonomic groups, from large bees and butterflies to hoverflies, beeflies, beetles and ants. In addition, intrapopulation diversity of flower visitors was very high, with Hurlbert's PIE values around 0.84. These results indicate that the pollination system of *E. popovii* is very generalist, as found in other *Erysimum* species, such as *E. mediohispanicum*, *E. nevadense* and *E. baeticum* (Gómez et al 2007; Ortigosa & Gómez 2010). Self-compatible and/or pollinator generalist plants are less prone to pollen limitation than self-incompatible and/or specialist species (Ashman et al. 2004; Knight et al. 2005). Nevertheless, pollen limitation has been found in another *Erysimum* species with a similar pollination system (Gómez et al. 2010). In *E. popovii*, pollen limitation affected seed production rather than emergence rate. This outcome points to a quantitative rather than a qualitative pollen limitation (Aizen & Harder 2007). However, because inbreeding depression in self-compatible species is more pronounced in late stages of the life cycle (Husband & Schemske 1996), further studies would be needed to confirm this conclusion in *E. popovii*.

Resource reallocation from untreated to pollen-supplemented flowers is always a concern in PL experiments. To avoid confounding results related to resource reallocation, some studies have submitted whole individuals to control or experimental treatments (Baker et al. 2000). Although we could not follow this approach because *E. popovii* individuals often produce several hundred flowers, we used two complementary controls, one internal and the other external to detect potential reallocation (Wesselingh 2007; Gómez et al. 2010). We found that the reproductive output of the two control treatments was similar and consistent, suggesting that resource reallocation, if existed, was weak. In fact, under severe resource reallocation we would have consistently expected better performance by external rather than internal control flowers. However, this was not so, as only half of the populations showed this pattern. In addition, we found a high degree of congruence between our two measures of pollen limitation (PL_{IC} and PL_{EC}), which showed the same trend in 12 out of the 13 populations that could be statistically analyzed (Table 2). Finally, we found no differences between the two controls for seedling emergence rate.

Variation among populations in pollinator assemblage may produce inter-population differences in pollen movement patterns and pollen transfer effectiveness, because different types of flower visitors have different flower-handling behaviours and show important differences in the amount and quality of pollen deposited per visit (Gómez & Zamora 1999; Fenster & Dudash 2001; Cosacov et al. 2008). Consequently, several studies have found that plant populations differ in the intensity of pollen limitation as a consequence of differences in pollinator abundance and diversity (Baker et al. 2000; González-Varo et al. 2009, Gómez et. al. 2010). In *E. mediohispanicum*, a co-ocurring and ecologically similar species to *E. popovii*, pollinator diversity and abundance significantly affect pollen limitation (Gómez et al. 2010). Under this perspective, it is remarkable that none of the two variables were correlated with pollen limitation intensity in *E. popovii*. *E. mediohispanicum* was found to be pollen limited in 50% of the populations studied (Gómez et al. 2010), whereas *E. popovii* is pollen limited throughout most of its geographic range. We believe that this disparity between the two plant species is a consequence of differences pollinator visitation rate between both species. Pollinator visitation rates in *E. popovii* (mean±SD: 0.348 visits flower^{-1} hr^{-1}; Appendix D) are three times lower than those reported for *E. mediohispanicum* (mean±SD: 0.960 visits flower^{-1} hr^{-1}; Gómez et al. 2007). More important, some populations received even a lower amount of visits. Since *E. popovii* flowers use to last only one or two days, we believe that this difference in pollinator abundance at flowers may cause the strong pollen limitation observed in this plant species. These differences could be due to traits intrinsic to each species (e.g., *E. popovii* individuals produce fewer flowers than *E. mediohispanicum*; Blanca et al. 2009), or to extrinsic factors such as differences in co-blooming plant composition. Lay et al. (2011) have recently found *Erysimum capitatum* not to be pollen limited, despite having a pollinator visitation frequency similar to *E. mediohispanicum*. They argue that this could be due to *E. capitatum* pollinators being more effective than those of *E. mediohispanicum*.

In *E. popovii*, beefly abundance was associated to low intensity of pollen limitation. Beeflies are likely to be very efficient pollinators of *E. popovii* because, compared to most other flower visitors, they move frequently between plants and visit few flowers per individual. This kind of foraging behavior has also been observed on *E. mediohispanicum* (Gómez et al. 2011). This foraging pattern potentially maximizes deposition of allogamous pollen per visit, which may reduce abortion rate and decrease pollen limitation during predispersal stages (Vaughton & Ramsey 2010). Beeflies have been shown to be highly efficient pollinators in other plant species (Motten et al. 1981; Johnson & Dafni 1998; Kastinger & Weber 2001; Anderson et al. 2005; Koopman & Ayers 2005), including the con-specific *E. mediohispanicum* (Gómez et al. 2009; Gómez et al.

2011). These results show that the abundance of highly efficient pollinator groups, rather than the abundance of the overall flower visitor assemblage, may be the most important determinant of pollen limitation in some pollination systems (Lay et al. 2011).

Our experiments also demonstrate that some environmental variables, such as annual rainfall and bare soil cover, are associated with pollen limitation in *E. popovii*. Annual rainfall was positively related to pollen limitation. This is probably due to the fact that plants growing in areas with low rainfall are unable to respond to supplementary pollination by increasing their seed number. In other words, in populations with low rainfall, seed production is likely to be limited by water availability or by a combination of water availability and pollen (Haig & Westoby 1988). Bare soil cover had a marginally significant negative effect on pollen limitation. A potential explanation could be related to the fact that most bees nest underground in areas deprived of vegetation. Consequently, bare soil availability may enhance the abundance and diversity of bees and other ground-nesting pollinators (Potts et al. 2005). In addition, by promoting bee abundance, bare soil also may enhance beefly abundance, as most beeflies are parasitic on ground nesting bees and wasps (Boesi et al. 2009).

In sum, our study shows that *E. popovii* is pollen limited throughout most of its geographic range, and although visited by a diverse community of pollinating insects, the intensity of pollen limitation in this species is mostly attributable to a specific pollinator group, namely beeflies.

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APPENDICES

APPENDIX A. *Moran's I analysis of the spatial structure of pollen limitation on seed production (PL_{IC}: pollen addition vs internal control; PL_{EC}: pollen addition vs external control) across the Erysimum popovii populations studied. Significance of Moran's I based on 300 permutations.*

Distance class	n	Distance (m)	Moran's I	*P*
PL_{IC}				
1	26	1557.8	0.31	0.16
2	24	13963.3	0.24	0.23
3	24	43669.1	-0.12	0.44
4	24	54900.8	-0.32	0.09
5	24	61101.6	0.13	0.4
6	24	72001.6	-0.79	0.005
PL_{EC}				
1	26	1557.8	0.035	0.82
2	24	13963.3	-0.56	0.01
3	24	43669.1	0.06	0.59
4	24	54900.8	-0.07	0.64
5	24	61101.6	0.06	0.66
6	24	72001.6	-0.1	0.51

APPENDIX B. *Pearson's Correlation Coefficient (r) between Flower Visitor variables.*

	Flower visitation rate	Flower visitor diversity	Beefly rate	Butterfly rate	Large bee rate	Small bee rate	Other rate
Flower visitation rate	-	**r=-0.836 p<0.001**	**r=0.733 p=0.004**	r=-0.249 p=0.410	**r=0.898 p<0.001**	r=-0.466 p=0.109	r=-0.115 p=0.707
Flower visitor diversity		-	r=-0.379 p=0.200	r=-0.320 p=0.286	**r=-0.942 p<0.001**	r=-0.394 p=0.183	r=0.152 p=0.621
Beefly rate			-	r=-0.156 p=0.610	r=0.498 p=0.083	r=-0.166 p=0.587	r=-0.431 p=0.142
Butterfly rate				-	r=-0.317 p=0.291	r=-0.065 p=0.832	r=-0.339 p=0.256
Large bee rate					-	r=-0.434 p=0.138	r=-0.281 p=0.356
Small bee rate						-	r=-0.007 p=0.982
Other rate							-

APPENDIX C. *Pearson's Correlation Coefficient (r) between Population variables.*

	Population size	Altitude	Rainfall	Co-occurring	Woody plant	Bare soil cover	Rock cover
Population size	-	r=-0.468 p=0.107	r=-0.125 p=0.685	r=0.041 p=0.896	r=-0.327 p=0.275	r=0.234 p=0.442	r=0.298 p=0.322
Altitude		-	r=-0.009 p=0.977	**r=0.553** **p=0.050**	r=-0.035 p=0.911	r=0.086 p=0.779	r=-0.289 p=0.338
Rainfall			-	r=0.002 p=0.994	r=0.299 p=0.321	r=-0.468 p=0.107	r=0.138 p=0.652
Co-occurring flowering plants				-	r=-0.364 p=0.221	r=-0.006 p=0.985	r=-0.319 p=0.288
Woody plant cover					-	r=-0.263 p=0.385	**r=-0.695** **p=0.008**
Bare soil cover						-	r=0.389 p=0.189
Rock cover							-

APPENDIX D. *Flower visitation rate, species richness and Diversity of flower visitors to the 21 Erysimum popovii populations studied.*

Population	Flower visits observed	Flower visitation rate (visits/hr of observation)	Flower visitation rate (visits/flower /hr)	Flower visitor species	Flower visitor diversity (Hurbert's PIE)
Ep01	202	30.6	0.498	21	0.83
Ep02	199	27.0	0.258	22	0.94
Ep03	282	88.8	0.096	13	0.73
Ep04	288	55.8	0.096	24	0.82
Ep05	229	19.8	0.594	22	0.88
Ep07	201	26.4	0.450	13	0.78
Ep08	236	22.2	0.510	20	0.91
Ep09	265	66.0	0.138	13	0.62
Ep10	236	34.8	0.354	23	0.86
Ep13	212	43.2	0.198	16	0.85
Ep14	331	55.2	0.120	32	0.94
Ep15	207	25.8	0.564	28	0.94
Ep16	195	14.4	1.050	18	0.9
Ep19	217	24.0	0.516	29	0.92
Ep20	193	17.4	0.606	25	0.84
Ep21	325	72.0	0.078	15	0.67
Ep22	202	33.6	0.378	16	0.85
Ep23	307	40.8	0.210	20	0.87
Ep27	271	64.8	0.156	11	0.79
Ep28	268	30.6	0.366	21	0.79
Ep30	302	60.0	0.084	14	0.84
Total	5168	34.2	0.348	166	0.95

CAPÍTULO 3

GEOGRAPHIC VARIATION IN RECRUITMENT LIMITATION OF A NARROW ENDEMIC HERB

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Geographic variation in recruitment limitation of a narrow endemic herb

ABSTRACT

Factors limiting plant recruitment may vary geographically as a consequence of co-occurring spatial variation in biotic interactions and abiotic conditions. Analysing this spatial variation is essential to know the mechanisms influencing the distribution of plant species, to understand the geographical mosaic at regional and local scale and to design appropriate conservation strategies. In this study we experimentally explore geographic variation in the three main processes limiting recruitment (low seed production, poor seed dispersal, inadequate microsite availability) for the near-threatened endemic crucifer *Erysimum popovii*. To this effect, we conducted a seed supplementation experiment in 19 populations spanning throughout most of the distribution area of the species. Three seed supplementation treatments were applied to each population: no seed addition (control), seed addition at the estimated natural seed density, and seed addition at 10 x the estimated natural seed density. Natural seed density was estimated separately for each population. The predominant type of limitation in each population was modeled by MCMCglmm (Generalised Linear Mixed Models using Markov Chain Monte Carlo), and the effects of biotic and abiotic factors on the type of recruitment limitation using spatially-explicit MCMCglmms. Factors limiting recruitment varied across populations but not across regions. Six populations were limited by seed production, seven by seed dispersal, and six by microsite availability. Density of seeds produced per population, herbaceous cover and beefly abundance, were associated with increased probability of limitation by microsite availability. On the other hand, abundance and diversity of overall pollinators, and shrub cover were associated with increased probability of limitation by seed availability and dispersal efficiency. The geographic variation observed in our study results in a mosaic structure of the factors regulating *E. popovii* recruitment dynamics at the local and regional scales. Our study is the first to describe spatial variability in the factors limiting recruitment across the entire distribution area of a threatened plant species.

KEYWORDS: *Community dynamics · Microsite limitation · Plant conservation · Plant population · Reproductive ecology · Seed dispersal · Seed limitation · Seed production · Seedling recruitment · Shifting limitations hypothesis*

INTRODUCTION

Plant recruitment may be limited by source availability (= seed production), inadequate dispersal, and microsite availability (Eriksson & Ehlérn 1992, Müller-Landau et al. 2002, Schupp et al. 2002), or by a combination of these three factors (Nathan & Müller-Landau 2000, Turnbull et al. 2000, Münzbergová 2004, Acácio et al. 2007, Scott & Morgan 2012). Limitation by source availability occurs when the number of seeds produced in a population is insufficient to fill all suitable sites in the landscape (Müller-Landau et al. 2002, Salazar et al. 2012). Limitation due to seed dispersal happens when the seeds fail to reach all suitable sites (Müller-Landau et al. 2002). Aggregated seedling distribution, a negative correlation between seed/seedling establishment and distance from the main seed sources, as well as lack of establishment in distant but otherwise adequate sites provide evidence for this kind of limitation (Dalling et al. 1998, Beach & Halpern 2001, Jacquemyn et al. 2002, Franzén and Eriksson 2003). Finally, limitation by microsite availability occurs when there is a shortage of sites adequate for seed germination and establishment (Clark et al. 1998, Clark et al. 1999). Some sites may be favourable to seed germination but unfavourable to the establishment of adults (Shima & Ellner 1984, Pulliam 1988, Schupp 2005). Therefore, it may be necessary to quantify the reproductive success of adults rather than just seedling recruitment if we want to assess the true magnitude of microsite limitation (Clark et al. 1999).

To investigate the factors conditioning local population dynamics, plant ecologists often resort to experiments of seed supplementation (Primack & Miao 1992, Thompson and Baster 1992, Orth et al. 1994, Robinson et al. 1995, Burke & Grime 1996, Tilman 1997, Münzbergová & Herben 2005, Moore and Elmendorf 2006). When recruitment limitation is due to seed shortage, adding seeds above the natural threshold of seed abundance should result in more seedlings (Münzbergová & Herben 2005). When recruitment limitation is due to inefficient seed dispersal, the number of seedlings should increase after distributing seeds across the landscape without increasing their number (Müller-Landau et al. 2002). If recruitment is due to microsite limitation, supplementation experiments will either result in no increase in seedling establishment or in an initial increase followed by a plateau that will not be overcome by subsequent supplementation (Eriksson & Ehlén 1992, van del Meijden et al. 1992, Grieshop and Nowierski 2002).

At the community level, biotic interactions may also decisively affect recruitment depending on the level of saturation within the community and the competitive performance of the species (Foster & Tilman 2003). In non-saturated communities, recruitment is mostly limited by seed availability, whereas in saturated communities, biotic interactions and niche availability are probably the main limiting factors (Tilman 1988, Cornell 1993, Foster 2001). An increase in recruitment following seed supplementation of various species would provide evidence that the community area is not saturated and colonization is seed-limited rather than restricted by the availability of adequate microsites (Tilman 1997, Zobel et al. 2000, Lord & Lee 2001).

Factors affecting recruitment are probably distributed along continuous gradients across the landscape, and are not necessarily mutually exclusive (Münzbergova & Herben 2005). It is thus unlikely that the establishment of a species will be strictly limited by a single factor (Foster & Tilman 2003). Rather, the relative importance of seed availability, seed dispersal and microsite availability would vary spatially depending on variation of biotic interactions and abiotic conditions (Edwards & Crawley 1999, Huston 1999, Schupp et al. 2002, Austrheim & Eriksson 2003, Foster et al. 2004, 2009, Stevens et al. 2004). Therefore, it is expected that the predominant type of recruitment limitation in a given species will vary geographically depending on local conditions, a pattern predicted by the shifting limitations hypothesis (Foster et al. 2004, 2009, Eskelinen & Virtanen 2005). Understanding how recruitment limitation varies and which types of limitation are more common or geographically widespread would be very important to understand the mechanisms influencing the distribution of plant species (Moore & Elmendorf 2006). However, as far as we know, empirical information on geographical patterns of recruitment limitation is mostly lacking. The few studies addressing this topic do not encompass the entire distribution of the species studied and only explore spatial variation in the intensity of recruitment limitation, obviating differences in the type of recruitment limitation (Eriksson & Ehlérn 1992, Nathan & Müller-Landau 2000, Schupp et al. 2002).

In this study, we experimentally measured recruitment limitation in *Erysimum popovii*, an endemic herb from south-eastern Spain, across most of its distribution area. In addition, we explored the relationship between local environmental, population and pollination-related variables and the observed patterns of recruitment limitation. Our objectives were: 1) to determine to what extent recruitment in *E. popovii* populations is variously limited by seed production, seed dispersal, and adequate microsite availability in different parts of its distribution area; 2) analyse the spatial distribution of these limitation types; and 3) explore the influence of biotic and abiotic factors on recruitment limitation.

METHODS

STUDY SPECIES

Erysimum popovii Rothm. (Brassicaceae) is a biennial to perennial herb endemic to several mountain ranges in southern Spain (Granada, Jaen and Cordova provinces, Fig. 1). The species is included in the Red List of Vascular Flora of Andalusia as near-threatened (Cabezudo *et al*. 2005). It occurs in pasture patches and rocky outcrops surrounded by calcareous scrubland at altitudes between 700 and 2000 m. The climate throughout the area is continental-Mediterranean, with cold winters and dry, hot summers. Rainfall is mostly restricted to spring and autumn.

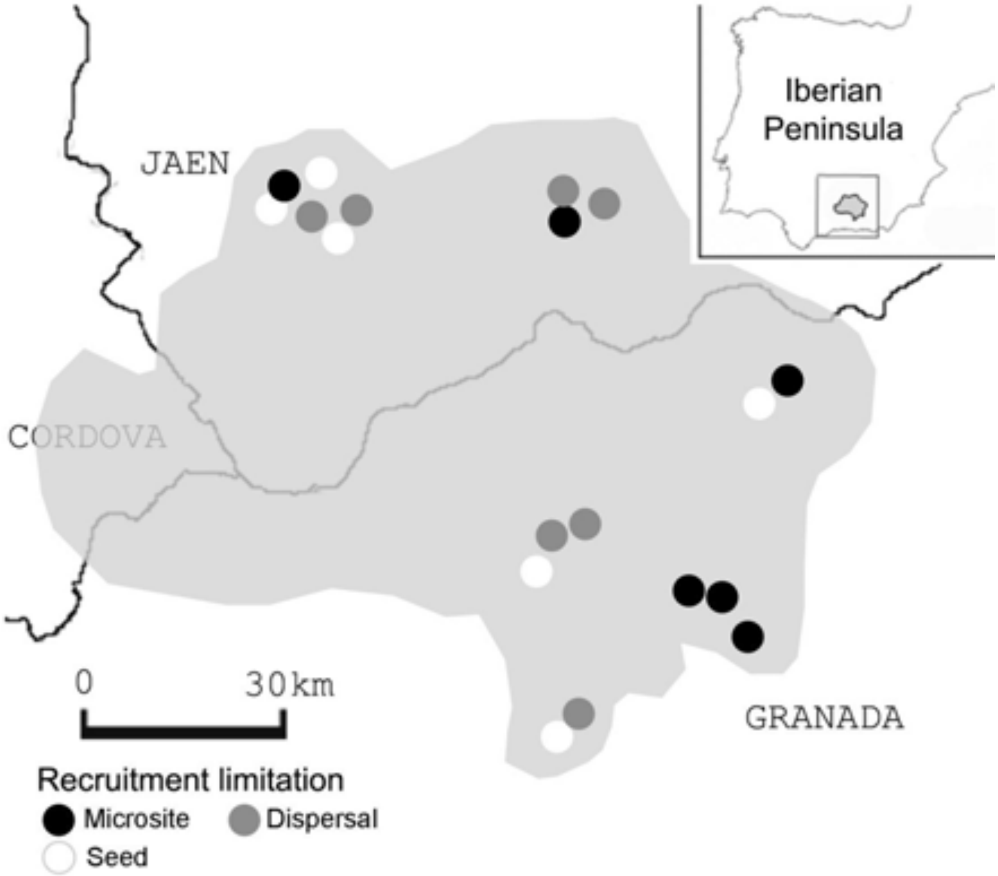

FIGURE 1: Erysimum popovii distribution (grey area) in SE Iberian Peninsula, with location of the 19 study populations and the type of recruitment limitation prevalent in each of them.

Erysimum popovii rosettes produce a few reproductive stalks that grow 20-40 cm tall and produce 2-80 purple flowers per plant in April-May (Blanca et al. 1992, Blanca et al. 2009). Dehiscent fruits mature in August and seeds are dispersed by gravity in October-December. As with other congeneric species (Gómez 2005), *E. popovii* plants are consumed by domestic and wild ungulates (Fernández & Gómez 2012). Seeds are sometimes predated by weevil larvae (personal observation). *Erysimum popovii* is a pollination-generalist plant, with flowers visited by a wide spectrum of insects. However, previous studies have revealed low pollinator visitation rates compared to other co-flowering species (Fernández et al. 2012, Fernández & Gómez 2012), resulting in pollen-limited seed yields (Fernández et al. 2012). *Erysimum popovii* lives in habitats subjected to disturbances (fragmentation, erosion, herbivory, etc.) (authors' personal observation).

STUDY AREA AND EXPERIMENTAL DESIGN

We selected 21 populations located in 6 mountain ranges (henceforth regions) covering the entire distribution area of the species. However, two populations had to be discarded due to heavy damage by livestock and wild boars, so we ended up using 19 populations (Fig. 1. Appendix A). Once field work was completed, two populations (Ep01, Ep19, from the Mencal region, Table 1, Appendix A) were provisionally reassigned by taxonomists to the related taxon *E. baeticum bastetanum*. However, and because taxonomic relationships between these two species are far from being resolved, we decided to keep these two populations in our study.

To estimate seed density, seed produced per m^2, in each population we selected 30 individual plants scattered across each site and collected their ripe fruits in September of 2008 and 2009. Seeds were counted in the laboratory using a magnifying glass. Seed production was then multiplied by population size (number of flowering individuals in each site) to obtain total seed yield of each population. Seeds from all plants within each population were pooled together. Seed supplementation was conducted in November 2009 and each site received seeds only from its own population. Seeds were sown in 0.25 m^2 permanent quadrats according to three treatments. In the first treatment seeds were sown at the estimated seed density of each population (T1). In the second treatment the number of seeds was incremented to 10 times the estimated seed density (T10), we use this treatment with several seed addition because the effects of seed addition are typically small. Clark et al. (2007) have pointed out that supplementing seeds at different densities enables verifying whether recruitment increases linearly with supplementation density or levels at high densities. The third treatment was a control with no seeds added (T0). In each population, we set up 10 randomly located replicates, each with three quadrats (one per treatment) placed side by side. From December 2010 to February 2011 we counted plants (by then pre-adult rosettes to adults) growing in the quadrats. Recruitment estimates were based on these counts, since these individuals had survived damage by ungulates and the intense summer drought typical of Mediterranean environments (Turnbull et al. 2000, Rey et al. 2006).

Following Münzbergová and Herben (2005), we considered higher recruitment in T_1 than T_0 as evidence of seed dispersal limitation, and higher recruitment in T_{10} than in T_1 as evidence of seed production limitation. No differences in recruitment between treatments were considered to reflect microsite limitation. Higher recruitment in T_{10} than T_1 together with higher recruitment in T_1 than T_0 was considered as evidence of a combined effect of seed dispersal and seed production (Münzbergová & Herben 2005).

BIOTIC AND ABIOTIC FACTORS AFFECTING RECRUITMENT

In each population we measured a series of environmental and population variables, as well as several variables related to the local pollination environment that could influence recruitment. Environmental variables were slope, percent shrub cover and percent herbaceous cover. Slope was measured with a clinometer. The two cover variables were measured along three 25 m x 2 m transects by noting cover type in 3 contact points every 0.5 m. We scored microhabitat type in the centre and at the two edges of the transect width (González-Megías et al. 2008). Slope may influence dispersal distance because seeds are dispersed by gravity in *E. popovii*. On the other hand, microhabitat type may affect microsite limitation. Herbaceous plants could compete with *E. popovii* for suitable microsites, and shrubs could have a nurse effect, thus increasing suitable microsite availability, as documented in other co-occurring *Erysimum* species (Gómez 2005).

We used population size (number of adult individuals) and seed density (seeds produced per m^2) as population variables. Population size was estimated by counting all flowering individuals in spring of 2008. Seed density was estimated by multiplying plant density (plants flowering per m^2) times seed production per plant in each population.

We considered three variables related to pollinators: pollinator diversity, pollinator abundance (insect flower^{-1} minute^{-1}) and beefly abundance (beefly flower^{-1} minute^{-1}). A relationship between these three variables and seed production has been previously found in *Erysimum* species, including *E. popovii* (Gómez et al. 2007, Perfectti et al. 2009, Gómez et al. 2010, Fernández & Gómez 2012, Fernández et al. 2012), with potential consequences on seed limitation. Pollinators other than bee-flies were not considered separately because of their lesser role as pollinators of *E. popovii* (Fernández et al. 2012). To measure these variables we conducted pollinator surveys in each population during the springs of 2008 and 2009. Counts were done on several days throughout the flowering period from 11:00 am to 17:00 pm (Fernández et al. 2012). We recorded a total of 4767 flower visits (193–331 visits per population). Pollinator diversity was estimated using the Hurlbert's PIE index (EcoSim 7, Gotelli and Entsminger 2009). This index measures the probability that two randomly sampled individuals from the community belong to different species.

STATISTICAL ANALYSIS

We first determined the predominant type of limitation in each study population by means of Generalised Linear Mixed Models using Markov Chain Monte Carlo techniques (MCMCglmm), which allows multi-trait models and random-effect variance structures to be specified (Hadfield 2010). We compared recruitment of paired treatments (T_0 versus $T_{1,}$ T_0 versus $T_{10,}$ and T_1 versus T_{10}), including treatment and replicate as fixed and random factors, respectively. Because seedling count matrices contained many zeroes, we considered a zero-inflated Poisson distribution to provide the best fit to our data.

Spatial variation in recruitment limitation was also explored with MCMCglmms. In these models we included the number of seedlings per treatment as a dependent variable. Region and population were included as fixed and random factors respectively, since all regions, but not all populations, in which *E. popovii* occurs were sampled. We assessed the significance of each independent variable by comparing the fit of models with and without each variable. To evaluate the goodness of fit of our models, we used the Deviation Information Criteria (DIC), whose value decreases with increasing fit. Additionally, we analyzed the spatial autocorrelation of the different types of recruitment limiation with Moran's I coefficient at different distance classes (Rangel et al. 2010). The number of distance classes was established by the default function of the software (SAM v. 4.0, Rangel et al. 2010).

The effects of biotic and abiotic factors on the type of recruitment limitation were explored using spatially-explicit MCMCglmms (Hadfield 2013). In these models, the dependent variable was the type of recruitment limitation, considered as a non-ordered categorical variable with two levels (seed + dispersal limitation versus microsite limitation). Independent variables with negative posterior mean values are associated to higher likelihood of microsite limitation, whereas those with positive values are associated to higher probability of seed and dispersal limitation. Because the distribution of *E. popovii* populations is strongly aggregated (Fernández *et al.* 2012, Fernández & Gómez 2012), we controlled for the spatial location of the populations. To this effect, the models included the spatial distance matrix of the plant populations as a random variance structure (Hadfield 2010).

The prior used in all MCMCglmm models was the inverse Wishart for both the units (residual) and variance (random element). This is a universal prior that works adequately both for categorical and binomial dependent variables as well as for continuous variables (Hadfield 2010). In addition, we run 500000 iterations of Montecarlo algorithm based on Markov's chains. All MCMCglmms were performed with the R package MCMCglmm (Hadfield 2010, 2013).

RESULTS

The recruitment of *E. popovii* was very low across its area of distribution. Natural recruitment (T_0) in the studied populations ranged from 0 to 5.14 recruited individuals per m^2, although it was zero or close to zero in most populations (mean ± SE = 0.75 ± 0.46, Table 1). When pooling together the results of all populations, we found that seed supplementation resulted in increased recruitment both in T_1 (mean ± SE = 1.17 ± 0.74) and T_{10} (1.56 ± 0.94) treatments. The magnitude of this increase, however, was very small, even reaching negative values in some populations. Of the 19 populations studied, six were limited by seed production with no differences between T_0 and T_1 and significant differences between T_1 and T_{10} ($T_0 = T_1$, $T_1 < T_{10}$), seven were limited by seed dispersal, showing significant differences between T_0 and T_1 and T_1 and no differences between T_{10} ($T_0 < T_1$, $T_1 = T_{10}$), and six were limited by the availability of suitable microsites, showing neither differences between T_0 and T_1 nor between T_1 and T_{10} ($T_0 = T_1$, $T_1 = T_{10}$) (Fig. 1, Table 1, Appendix B).

The MCMCglmms indicate that there were differences in *E. popovii* recruitment limitation among populations but not among regions. Model B, including only treatment as independent variable, was the model with the lowest DIC (Table 2). The type of recruitment limitation showed no spatial autocorrelation (Appendix C).

Region	Population	Seed density	T_0 recruitment	T_1 recruitment	T_{10} recruitment	Significant differences between treatment	Type of limitation
Mencal	Ep 01	7	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	$T_0 = T_1 = T_{10}$	Microsite
	Ep 19	102	0.00 ± 0.00	0.00 ± 0.00	0.44 ± 0.44	$T_0 = T_1 < T_{10}$	Seed
Sierra Nevada	Ep 03	21	0.00 ± 0.00	0.00 ± 0.00	0.89 ± 0.59	$T_0 = T_1 < T_{10}$	Seed
	Ep 27	72	0.57 ± 0.57	0.00 ± 0.00	0.57 ± 0.57	$T_0 < T_1 = T_{10}$	Dispersal
La Peza	Ep 04	18	2.80 ± 1.20	6.00 ± 3.88	8.80 ± 3.09	$T_0 = T_1 = T_{10}$	Microsite
	Ep 23	269	5.14 ± 2.72	4.57 ± 2.21	2.86 ± 1.44	$T_0 = T_1 = T_{10}$	Microsite
	Ep 09	20	1.60 ± 0.89	1.60 ± 0.89	1.60 ± 0.89	$T_0 = T_1 = T_{10}$	Microsite
Sierra de Jabalcuz	Ep 05	114	0.00 ± 0.00	0.00 ± 0.00	0.80 ± 0.53	$T_0 = T_1 < T_{10}$	Seed
	Ep 08	51	0.00 ± 0.00	0.80 ± 0.53	1.20 ± 0.85	$T_0 < T_1 = T_{10}$	Dispersal
	Ep 10	41	0.00 ± 0.00	0.89 ± 0.59	1.33 ± 1.33	$T_0 < T_1 =T_{10}$	Dispersal
	Ep 14	24	0.00 ± 0.00	0.00 ± 0.00	0.40 ± 0.40	$T_0 = T_1 < T_{10}$	Seed
	Ep 15	65	0.00 ± 0.00	0.00 ± 0.00	0.80 ± 0.53	$T_0 = T_1 < T_{10}$	Seed
	Ep 16	26	2.40 ± 1.60	1.20 ± 1.20	2.80 ± 1.98	$T_0 = T_1 = T_{10}$	Microsite
Sierra Arana	Ep 13	24	0.00 ± 0.00	0.80 ± 0.80	0.40 ± 0.40	$T_0 < T_1 = T_{10}$	Dispersal
	Ep 20	26	0.40 ± 0.40	0.40 ± 0.40	2.00 ± 0.89	$T_0 = T_1 < T_{10}$	Seed
	Ep 21	45	0.40 ± 0.40	1.60 ± 0.65	1.20 ± 0.61	$T_0 < T_1 = T_{10}$	Dispersal
Sierra Mágina	Ep 07	37	0.40 ± 0.40	0.40 ± 0.40	1.60 ± 1.60	$T_0 = T_1 = T_{10}$	Microsite
	Ep 28	51	0.00 ± 0.00	1.20 ± 0.61	0.80 ± 0.53	$T_0 < T_1 = T_{10}$	Dispersal
	Ep 30	17	0.57 ± 0.57	2.86 ± 1.89	1.14 ± 1.14	$T_0 < T_1 = T_{10}$	Dispersal

TABLE 1. Seed density (seeds per m^2*), recruitment (recruited plants ± 1 SE per* m^2*) following treatments (*T_0*,* T_1*, and* T_{10}*) and type of recruitment limitation in the 19 Erysimum popovii populations studied. Significant differences between treatments and the resulting type of recruitment limitation in each population are also shown. "<" represents significant differences (*$p<0.05$*) between treatments in recruitment and "=" lack thereof (see Appendix B for statistical details).*

	Factors		
Models	Fixed	Random	DIC
A	Treatment		610.3658
B	Treatment	Population: Region	**595.1105**
C	Treatment + Region	Population : Region	598.3715
D	Treatment *Region	Population : Region	599.407

TABLE 2. Results of the Bayesian generalized linear mixed model testing spatial variation in E. popovii recruitment. Model A: model with only Treatment as fixed factor. Model B: model with Treatment as fixed factor and Population within Region as random factor. Model C: model with Treatment and Region as fixed factors and Population within Region as random factor. Model D: model with Treatment, Region and the interaction between both as fixed factors and Population within Region as random factor. DIC: Deviance Information Criterion.

BIOTIC AND ABIOTIC FACTORS AFFECTING RECRUITMENT LIMITATION

The spatially-explicit MCMCglmms indicate that several factors affected the type of recruitment limitation (microsite *versus* seed+dispersal) in *E. popovii* populations (Table 3). Three factors: seed density, herbaceous cover and beefly abundance, were associated to an increase in the probability of limitation by microsite availability (Table 3). On the other hand, three factors, pollinator abundance, pollinator diversity and shrub cover, were associated to an increase in the probability of limitation by seed availability and dispersal efficiency (Table 3). Thus, populations with high shrub cover were limited by seed but not by microsite availability, whereas populations with high herbaceous cover were limited by microsite availability. Surprisingly, populations with high pollinator diversity and abundance tended to be limited by seed rather than microsite availability. However, populations with high beefly visitation were limited by microsite availability rather than by seed production (Table 3).

Variables	Posterior mean	l-95% CI	u-95% CI	pMCMC
Intercept	-3169.231	-6483.174	-320.729	0.0244
Population size (# plants/population)	-0.430	-0.912	0.032	0.0734
Seed density (seeds/m^2)	-6.714	-11.986	-1.132	0.0018
Pollinator abundance (visits/min flower)	520.096	3.648	1065.138	0.0448
Pollinator diversity (Hurlbert's PIE)	4574.730	1178.851	8622.061	0.0016
Beefly abundance (visits/min)	-16.071	-32.411	-1.451	0.0306
Slope (%)	-8.817	-16.604	0.408	0.0832
Shrub cover (%)	3395.706	795.731	6264.707	0.0017
Herbaceous cover (%)	-4196.367	-6428.864	-2658.764	0.0001

TABLE 3. Results of spatially explicit Bayesian generalized linear mixed model testing the relationship between several factors and E. popovii recruitment limitation. Posterior means, 95% credible intervals (CI) and Bayesian P values are shown for each factor. Independent variables with negative posterior mean values are associated to a higher probability of microsite limitation, whereas those with positive values are associated to higher probability of seed production and dispersal limitation. DIC= 0.254.

DISCUSSION

Our study demonstrates that, at the species level, recruitment is limited by different processes (low seed production, poor dispersal, low microsite availability) in different parts of its distribution area. Importantly, limitation type was not autocorrelated, and differences in limitation type were stronger among populations than among regions. Therefore, the different causes determining recruitment limitation exhibit a mosaic structure in *E. popovii* populations across its distribution area. Spatial variation in recruitment limitation has been found in other Mediterranean plants (Herrera et al. 1994, Jordano & Herrera 1995, Gómez et al. 2003, García et al. 2005, Garrido et al. 2005, Lázaro et al. 2006, Acácio et al. 2007, Gómez-Aparicio 2008). However, most of these studies do not encompass the entire distribution of the species studied. More importantly, these studies explored spatial variation in the intensity of recruitment limitation, whereas our study addresses differences in the type of recruitment limitation. As far as we know, ours is the first study to address this issue.

We found that about one-third (31.6%) of the studied populations had their recruitment limited by seed availability, indicating that low recruitment in these populations is mostly a consequence of low seed production. This finding can be related to the small size of most *E. popovii* populations and the near threatened status of the species. The low number of seeds produced per population agrees with the significant pollen limitation experienced by this species in many populations (Fernández et al. 2012). Thus, low seedling recruitment in *E. popovii* could be, in part, a delayed consequence of poor pollinator performance. Surprisingly, however, limitation by seed production was higher in populations with higher pollinator abundance and diversity. Although counterintuitive, this result is consistent with previous studies finding no relationship between pollinator abundance or diversity and *E. popovii* reproductive output (Fernandez et al. 2012), suggesting that the activity of most *E. popovii* pollinators does not cause an increase in population-level seed production. In this respect, we found that recruitment limitation by seed production was less probable in populations with high abundance of beeflies. Beeflies are very efficient pollinators of *E. popovii* (Fernández et al. 2012), as well as other species (Motten et al. 1981, Johnson & Dafni 1998, Kastinger & Weber 2001, Koopman & Ayers 2005). That is, our finding may suggest that populations with many high-efficiency pollinators are less prone to be limited by seed availability than populations were most flower visitors are poor pollinators.

Recruitment was limited by seed dispersal in more than one-third (36.8%) of the studied populations and this may be related to *E. popovii* population structure. As mentioned, plant density (mean ± SD: 0.13 ± 0.03 individuals per m^2) and abundance (537 ± 114 individuals) is low in most populations (Fernández et al. 2012). In addition, adult plants show an aggregated distribution (personal observation), suggesting inability of seeds to colonize many suitable microsites. Gómez (2007) found that dispersal distance was extremely small —less than 50 cm— in *E. mediohispanicum*, a congener inhabiting similar habitats in the same region. We presume that *E. popovii* seed dispersal distance will be also very small, mostly because this species is shorter than *E. mediohispanicum*, and dispersal distance is positively associated with the height of the flowering stalks (Gómez 2007). Seed rain is not homogeneous across an entire population, but presumably concentrates close to reproductive individuals, with the likely appearance of intraspecific competition for resources among emerging seedlings (Eriksson & Ehlén 1992). Consequently, within a population, seed production limitation is likely to be more important away from reproductive individuals, whereas microsite limitation is likely to be more important in sites close to reproductive individuals. It is important to note that failure to reach a given location may be due to insufficient seed production or to poor dispersing patterns. Thus, even at a fixed seed production rate, the balance between the two types of limitation may change as a function of dispersal ability (Harper 1977, Nathan & Müller-Landau 2000, Satterthwaite 2007)

Once a seed has reached a given microsite, low recruitment may be explained by high seedling mortality caused by summer drought. Seed germination of *E. popovii* in the field is about 10%, but most seedlings die during the summer (unpublished data). In Mediterranean environments, summer drought is a main mortality factor, both in other *Erysimum* species (Gómez 2005) and in other herbaceous and woody species (Fernández et al. 1993, Castro et al. 2004, Crone & Lesica 2006, Giménez-Benavides et al. 2007, Venable 2007, Pérez-Ramos et al. 2010). In addition, we observed some damage by ungulates. Mammalian herbivory is another common cause of low recruitment in many plant communities of the Mediterranean area (Crawley 1989, Hanley and Fenner 1998, Herrera 2000, Baraza et al. 2007, Fernández & Gómez 2012). On the other hand, grazing may have a positive effect on colonization by certain plant species, especially in habitats with low-productivity, as it reduces the density of abundant species, thus lowering competitive exclusion (Grime 1973). In our study area, grazing by native ungulates fluctuates seasonally, but grazing by livestock is prevalent throughout most of the year. These herbivores consume reproductive stalks bearing fruits and flowers (Gómez 2005, Fernández & Gómez 2012), and may damage seedlings and rosettes not only through consumption but also through trampling, thus affecting both plant abundance and spatial distribution (Vargas et al. 2002, Gómez et al. 2003, Albon et al. 2007, Uytvanck et al. 2010). These two mortality factors may cause recruitment to be limited by microsite availability, because plants can only establish in microsites unaffected by ungulates and summer drought. Throughout the study, we observed that adult plants often grow close to or underneath the canopy of several species of shrubs (*Berberis hispanica*, *Rosmarinus officinalis*, *Thymus vulgaris*, *Thymus mastichina*, *Cytisus scoparius*). These shrubs are likely to act as nurse plants, protecting *E. popovii* individuals from water stress and, most important, ungulates, as has been found in other *Erysimum* (Gómez 2005). This type of facilitation is common in habitats with harsh conditions, such as our study area, where summer drought is severe (Brooker & Callaghan 1998), and in which shrubs provide a buffered microenvironment (Pugnaire et al. 2001, Rey et al. 2004, Gómez-Aparicio et al. 2004). As opposed to shrub cover, herbaceous cover was associated to an increase in microsite limitation. This effect is probably mediated by interspecific competition (Schoener 1983, Brandt & Seabloom 2012), and its intensity increases with increased seed production.

CONCLUSIONS

By studying recruitment across the entire distribution of *E. popovii* we have shown that, rather than limited by one single factor, there is a mosaic structure in the recruitment dynamics of this species. The type of recruitment limitation varies between populations and regions as a consequence of concomitant variation in habitat characteristics and biotic interactions. This outcome agrees with the main predictions of the shifting limitations hypothesis (Foster et al. 2004, Eskelinen & Virtanen 2005), and highlights the importance of considering potential geographic variation in the type of limitation affecting plant recruitment. Our results may have important consequences for the implementation of conservation policies. Information on recruitment processes and their driving factors at several spatial scales would be very useful to design successful plant conservation strategies, being as result in one scale cannot be extrapolated to other scales (Münzbergová 2004). In areas limited by seed number or seed dispersal, restoration practices should be directed toward increasing the number of seeds in the population (Seabloom *et al.* 2003). In contrast, in areas limited by the availability of suitable microsites, efforts should be directed towards selecting appropriate locations for seed addition (Moore & Elmendorf 2006). Knowledge of the biotic and abiotic conditions in each site would help establish the most likely type of limitation at a local scale. This approach would greatly improve the success of conservation plans for many threatened and endangered plant populations.

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APPENDICES

APPENDIX A. *Population, environmental and pollinator variables of each study population. xy: UTM coordinates, Altitude: meters above sea level, Precipitation: Accumulated annual precipitation in l per* m^2*, Slope: % slope, Seed density: Number of seeds per* m^2*, Population size: Number of adult E. popovii individuals, Pollinator abundance: visits per minute and flower, Pollinator diversity: Hurlbert's PIE index, Beefly abundance: Visits per minute and flower, Shrub cover: Percentage of surface covered by shrubs, Herbaceous cover: Percentage of surface covered by herbaceous plants.*

Region	Population	x	y	Altitude	Precipitation	Slope	Seed density	Population size	Pollinator abundance	Pollinator diversity	Beefly visitation rate	Shrub cover	Herbaceous cover
Mencal	Ep 01	487836	4155063	982	493.5	43	7	600	0.51	0.83	0.45	0.14	0.17
	Ep 19	483826	4151106	1188	496.7	35	102	420	0.40	0.92	7.22	0.15	0.17
Nevada	Ep 03	456751	4109531	1596	510.2	58	21	280	1.48	0.74	44.84	0.20	0.13
	Ep 27	457430	4109448	1486	540.6	25	72	340	1.08	0.79	48.24	0.37	0.33
Peza	Ep 04	467701	4118300	1503	577.0	0	18	300	0.93	0.82	19.74	0.12	0.23
	Ep 23	472949	4119278	1720	602.6	35	269	750	0.68	0.87	16.53	0.56	0.08
	Ep 09	472834	4119460	1693	609.1	30	20	130	1.10	0.62	6.75	0.47	0.25
Jabalcuz	Ep 05	425545	4177799	1321	725.6	60	114	420	0.33	0.88	3.74	0.05	0.27
	Ep 08	425519	4177931	1324	726.7	43	51	400	0.37	0.91	2.25	0.05	0.36
	Ep 10	424122	4177754	1060	737.3	62	41	450	0.58	0.86	12.30	0.00	0.27
	Ep 14	426894	4180210	970	689.0	0	24	120	0.92	0.94	11.33	0.12	0.48
	Ep 15	425148	4179387	1080	648.0	35	65	650	0.43	0.94	4.50	0.09	0.31
	Ep 16	425152	4179487	1060	640.5	35	26	150	0.25	0.90	2.94	0.01	0.10
Arana	Ep 13	454337	4131901	1738	642.4	35	24	370	0.72	0.85	0.00	0.01	0.43
	Ep 20	451048	4130531	1270	640.6	36	26	350	0.29	0.84	0.36	0.06	0.09
	Ep 21	452839	4131133	1498	675.5	47	45	280	1.20	0.67	12.00	0.27	0.14
Mágina	Ep 07	453888	4176684	2000	549.6	38	37	500	0.44	0.78	11.87	0.24	0.02
	Ep 28	455789	4176686	1661	559.4	11	51	1650	0.52	0.79	14.42	0.37	0.19
	Ep 30	453436	4176812	1898	582.7	35	17	2050	1.00	0.84	17.40	0.32	0.27

APPENDIX B. *Results of Generalised Linear Mixed Models using Markov Chain Monte Carlo techniques (MCMCglmm), comparing recruitment of paired treatments (*T_{10} *versus* $T_{1,}$ T_{10} *versus* $T_{1,}$ *and* T_1 *versus* T_0*). "-" indicates populations with no recruitment in any of the treatments.*

Population	Posterior Mean	l-95% Credible Interval	u-95% Credible Interval	Bayesian p-value
Comparison between T_{10}-T_1				
Ep01	-	-	-	-
Ep03	98.61	62.55	133.71	<0.001
Ep04	0.33	-1.55	2.19	0.720
Ep05	-15.17	-17.77	-13.84	<0.001
Ep07	4.75	-1.21	11.82	0.108
Ep08	1.21	-0.50	2.54	0.423
Ep09	0.19	-2.25	2.24	0.953
Ep10	1.74	-7.00	8.95	0.328
Ep13	-0.82	-9.544	8.21	0.608
Ep14	166.00	57.34	261.49	0.001
Ep15	-31.45	-43.33	-14.41	<0.001
Ep16	-1.07	-19.25	12.14	0.939
Ep19	1.68	1.54	1.86	<0.001
Ep20	2.29	0.45	4.31	0.022
Ep21	-0.17	-2.62	2.14	0.892
Ep23	-0.38	-1.84	1.08	0.740
Ep27	-41.16	-107.30	46.55	0.562
Ep28	0.61	-1.01	2.38	0.292
Ep30	-4.17	-118.03	66.13	0.841
Comparison between T_1-T_0				
Ep01	-	-	-	-
Ep03	-	-	-	-
Ep04	0.32	-1.89	2.56	0.731
Ep05	-	-	-	-
Ep07	2.81	-277.17	239.93	0.463
Ep08	7.19	3.82	14.19	0.004
Ep09	-0.26	-1.84	2.01	0.547
Ep10	-9.13	-16.66	-2.17	<0.001
Ep13	32.22	14.54	60.74	<0.001
Ep14	-	-	-	-
Ep15	-	-	-	-
Ep16	-0.57	-8.01	7.31	0.780
Ep19	-	-	-	-
Ep20	-9.41	-19.20	6.60	0.589
Ep21	1.83	1.82	1.85	<0.001
Ep23	-0.09	-1.85	1.72	0.887
Ep27	2.19	0.70	4.42	<0.001
Ep28	31.59	11.27	41.88	<0.001
Ep30	2.40	0.41	4.13	0.033
Comparison between T_{10}-T_0				
Ep01	-	-	-	-
Ep03	21.78	4.86	32.99	<0.001
Ep04	0.68	-0.29	1.87	0.781
Ep05	-15.01	-15.89	-12.97	<0.001
Ep07	-4.45	-90.13	23.83	0.498
Ep08	19.75	9.65	30.32	0.001
Ep09	0.361	-1.22	1.91	0.895
Ep10	31.47	3.41	53.76	<0.001
Ep13	20.82	2.36	48.94	0.015
Ep14	-65.25	-86.63	-39.70	<0.001
Ep15	2.91	1.20	3.91	<0.001
Ep16	-0.62	-6.21	5.59	0.752
Ep19	-12.33	-16.00	-9.59	<0.001
Ep20	2.08	-0.72	6.48	0.145
Ep21	3.78	1.54	7.62	0.006
Ep23	-0.34	-2.02	1.50	0.627
Ep27	-2.80	-6.07	-1.65	<0.001
Ep28	68.22	36.61	92.06	<0.001
Ep30	2.18	-3.11	5.91	0.310

APPENDIX C. *Spatial autocorrelation (expressed as Moran`s I) of recruitment following seed supplementation treatments (T0, T1, and T10) at different distances.*

Treatment	**Distance class**	**Moran`s I**	**Standart Error**	**P value**
T_{10}	1	0.014	0.133	0.599
	2	-0.258	0.146	0.166
	3	-0.054	0.128	0.987
	4	-0.099	0.149	0.772
	5	-0.047	0.126	0.945
	6	-0.206	0.145	0.3
	7	0.013	0.142	0.629
T_1	1	0.163	0.144	0.129
	2	-0.425	0.132	**0.005**
	3	-0.218	0.151	0.284
	4	-0.135	0.128	0.535
	5	0.109	0.153	0.282
	6	-0.214	0.134	0.237
	7	-0.103	0.13	0.714
T_0	1	0.153	0.142	0.14
	2	-0.377	0.136	**0.018**
	3	-0.039	0.146	0.911
	4	0.032	0.134	0.511
	5	-0.173	0.147	0.424
	6	-0.073	0.137	0.901
	7	-0.194	0.133	0.299

CAPÍTULO 4

ADVANTAGES AND DRAWBACKS OF LIVING IN PROTECTED AREAS: THE CASE OF THE THREATENED *ERYSIMUM POPOVII* (BRASSICACEAE) IN SE IBERIAN PENINSULA

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Advantages and drawbacks of living in protected areas: the case of the threatened *Erysimum popovii* (Brassicaceae) in SE Iberian peninsula

ABSTRACT

Protected areas help to develop regional and global strategies for the conservation of biodiversity. However, human-related activities, such as grazing, tourism, hardwood extraction, etc., may cause the decline of some species. In this study, we investigate whether inhabiting protected areas affect the conservation of *Erysimum popovii*, a narrow endemic and endangered plant, in Mediterranean environments with traditional human activities. We surveyed its entire distribution area in SE Spain, and located 31 populations, sixteen within Nature Parks (Category V of the IUCN) and fifteen in non protected areas. In each population we measured several variables related to habitat composition, pollinator community, herbivory damage and population viability. Our results show that populations in protected areas inhabit localities at higher altitudes and with more shrub cover. Most important, the abundance of a very effective pollinator type, beeflies, was significantly higher in protected populations. Curiously, damage by ungulates was also more intense in protected areas. The negative effect of herbivory offsets any positive effect played by pollinators in protected areas, and consequently there were not differences in population size or reproductive output inside and outside protected areas. It seems that allowing traditional uses in protected areas may entail negative effects for some vulnerable plant species.

KEYWORDS: Endangered plants · IUCN categories · Nature Park · Pollination · Protected area · Shepherding

INTRODUCTION

Protected areas play a fundamental role in safeguarding species and natural habitats, especially those most vulnerable to anthropogenic disturbance (Telleria 2001; Angelstam & Andersson 2001; Chape et al. 2005; Araujo et al. 2007; Carvalheiro et al. 2010). In addition, protected areas help to develop regional and global strategies for the conservation of biodiversity (Glowka et al. 1994; Jackson & Gaston 2008). To serve their purpose, these areas should (a) ensure the maintenance of species and their ecological interactions in the short, middle and long terms; (b) be representative of the regional biodiversity of species and habitats; and (c) ensure habitat preservation (Church et al. 2000; Margules & Pressey 2000; Lindenmayer et al. 2006; Leroux et al. 2007; Branquart et al. 2008). To reach this objective, land managers should ensure the long-term viability of the species within protected areas, by preventing harmful practices and promoting beneficial ones. However, recent times have seen a shift in conservation policies towards allowing and even promoting the use of local resources in some protected areas, often invoking the preservation of cultural tradition (Naughton-Treves et al. 2005). Sometime, it is difficult to reach the balance between the preservation of some traditional uses (i.e. farming, livestock and grazing, etc.) and the maintenance and preservation of biological diversity (IUCN 1994). It has been repeatedly reported that the occurrence of human-related activities within protected areas, such as grazing, tourism, hardwood extraction, etc., may cause a decline of species living in these areas (Caro 1999; Kerbiriou et al. 2009). A profound knowledge of the benefits and costs of living within protected areas on endangered species is crucial to establish conservation priorities, develop accurate management strategies and allow for sustainable development (Carroll & Groom 2006; Primack 2008).

Many factors shape the long- and short-term dynamics of rare plant populations. The structure and configuration of the habitat have been proven to mediate the population viability of many threatened species (Oostermeijer et al. 1998; Berg et al. 2002; Drupé & Ehrlén 2002). For this reason, many conservation practices have focused in improving the habitat of rare and endangered species (Bragazza 2009). Biotic interactions, both mutualistic and antagonistic, are also important factors determining the reproductive success and the recruitment rate of many rare plants. Pollinators are a main mutualistic organisms influencing plant distribution and population growth (Aizen & Feisenger 1994; Pauw & Bond 2011). Increased pollen limitation, reduced seed set and even seedling recruitment are often related to low pollinator abundance (Baker et al. 2000; Cosacov et al. 2008; González-Varo et al. 2009; Gómez et al. 2010). Pollinator composition and diversity have been also shown to influence reproductive success in some plant species (Waser 1978; Motten et al. 1981; Snow 1982; Steffan-Dewenter et al. 2001; Waites & Agren 2004). This is so because different pollinators usually differ in pollination effectiveness and may have different effects on plant fitness (Klein et al. 2002; Gómez et al. 2007; Perfectti et al. 2009). In fact, pollinator diversity is often associated with an increase in the probability of being visited by effective pollen vectors (Perfectti et al. 2009).

Damage by herbivores, on the other hand, is also a very important factor limiting recruitment and population growth of many plant species (Crawley 1989; Baraza et al. 2007). Herbivores may cause an arrest in plant recruitment to the point of preventing regeneration (Crawley 1997). Herbivores may affect plant abundance and spatial distribution not only via direct consumption but also via trampling (Gómez et al. 2003; Albon et al. 2007; Uytvanck et al. 2010). Consequently, excessive grazing may affect the conservation of endemic and endangered species (Simon et al. 2001).

In this study we investigate whether inhabiting protected areas benefits plants in Mediterranean environments with traditional human activities, especially grazing. We use as study system athreatened wallflower, *Erysimum popovii,* endemic to mountain areas of Andalusia (southern Spain). Previous studies with this and other conspecific co-occurring wallflower species have demonstrated that both pollinators and mammalian herbivores strongly influence plant reproduction and population growth (Gómez 2005a, 2005b; Gómez et al. 2007, 2010; Fernández et al. 2012). Here, we explore whether these interactions vary in intensity between protected and non protected areas. The specific goals of our study are:

(1) to determine the differences between protected and non protected areas inhabited by *E. popovii* in habitat characteristics.

(2) to quantify the abundance and diversity of pollinators in protected and non protected *E. popovii* populations.

(3) to estimate ungulate damage experienced by *E. popovii* in protected and non protected areas.

(4) to estimate differences in the population viability of *E. popovii* inhabiting protected and non protected areas.

(5) to suggest management strategies to enhance the effectiveness of protected areas for the conservation of *E. popovii* .

MATERIAL AND METHODS

STUDY SYSTEM

The Network of Protected Areas of Andalusia (RENPA) covers 18.4% of the Andalusia region surface and is composed of 130 areas belonging to six categories: National Parks, Nature Parks, Natural Reserves, Natural Monuments, Natural Places and Periurban Protected areas (Voth 2007). The most prominent category in Andalusia is Nature Parks, representing 83.43% of the total protected area (Consejería de Medio Ambiente 2007). The Nature Park corresponds to the Category V of the World Conservation Union classification (IUCN 1994). Category V is the most frequent category, accounting for 62% of the total protected surface in the Mediterranean region (López et al. 2007). This category is defined as an area with a distinctive aesthetic, ecological or cultural value as a consequence of the long-term interaction between people and nature (UICN 1994). Consequently, one of the main goals of this type of protected area is to safeguard the traditional use of local resources (López et al. 2007; Voth 2007).

Erysimum popovii Rothm. (Brassicaceae) is a narrow endemic plant from Southeastern Spain, included in the Red List of Andalusia Vascular Flora (Cabezudo et al. 2005). It inhabits mountain areas of the Baetic Mountain range (Granada, Jaen and Cordoba provinces; Blanca et al. 2009), from 900 to 2000 m. a.s.l., occupying rocky areas and gaps within shrublands. It is a biennial to short-lived perennial monocarpic herb, producing from a few to several hundred flowers on a variable number of reproductive stalks. Flowers are hermaphroditic and slightly protandrous, with four bright purple petals (Blanca et al. 1992; Blanca et al. 2009). Like other *Erysimum* species (Gómez 2005b), *E. popovii* requires insects as pollen vectors for full seed set. Flowers are visited by a diversity assemblage of insects composed mostly of large bees, beeflies, small bees and beetles (Férnandez et al. 2012). *E. popovii* cannot be pollinated by wind (unpublished observations from greenhouse experiments).

During 2007-2009 we surveyed the entire distribution area of *E. popovii*, and looked carefully for plant populations in all the localities reported as inhabited by the species, as well as in any other a priori suitable areas based on ecological and topographic characteristics (Blanca et al. 2009). We found 31 populations, 16 in Nature Parks and 15 in non protected area (Fig. 1). Some population are in Sierra Nevada mountains, which includes both one Nature and one National Park. However, all *E. popovii* populations in this mountain range are also located within the Nature Park. Two of the protected populations were excluded from our research because they suffered mechanical damage due to silvicultural treatments. Consequently, our study was conducted on 29 *Erysimum popovii* populations (Appendix A), spanning the entire geographic range of the species (Fig. 1). The overall area has a characteristic Mediterranean climate with cool wet winters and warm dry summers.

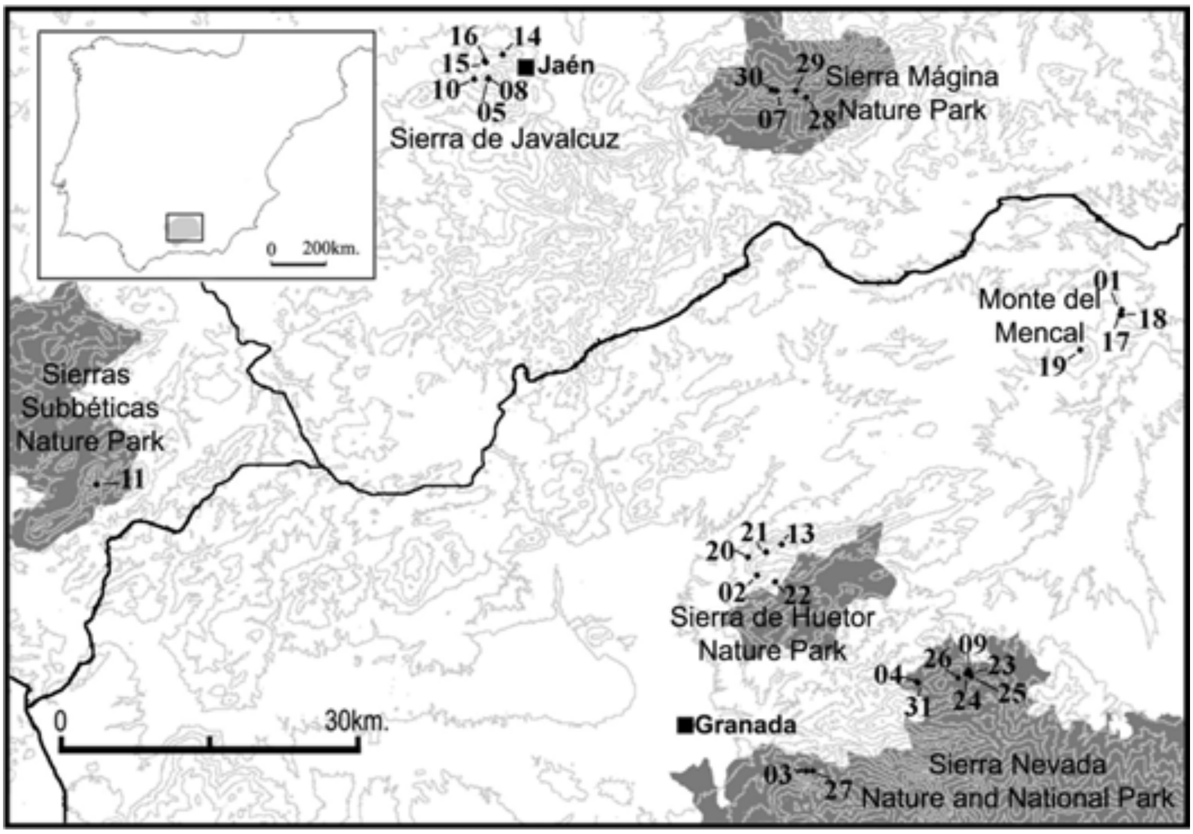

Figure 1. *Distribution area of Erysimum popovii (in gray in the Iberian peninsula map) and location of the 29 populations considered in this study. Protected areas in dark gray. Light gray lines are 25 m isoclines. Black lines represent the boundaries between Granada, Jaén and Córdoba provinces.*

HABITAT CHARACTERISTICS

Each of the 29 *Erysimum popovii* populations was characterized by quantifying the following variables: 1) Altitude, expressed as meters above the sea level. We used a GPS to estimate the altitude in the center of each population. 2) Co-occurring Flowering Plant Diversity, characterized with the Hurlbert's PIE Index. This index measures the probability that two randomly sampled units from a group belong to different species. It was calculated using the software EcoSim 7 (Gotelli & Entsminger 2009). 3) Co-occurring Flowering Plant Density, expressed as flowers per square meter. This and the previous variable were measured in spring of 2008, by establishing three transects of 10 x 2 meters and counting the number of individuals and flowers of plants blooming at the same time as *E. popovii*. 4) Habitat diversity, characterized with the Hurlbert's PIE Index of habitat composition. For this, in autumn of 2008, we laid three additional transects of 25 x 2 meters, and every 0.5 meters, scored microhabitat type in the center and at the two edges of the transect width. The microhabitat categories considered in this study were: Trees, Shrub, Scrub, Small scrubs, Perennial Herbs, Tussock Grasses, Dead Leaves, Dead Wood, Gravels, Rocks, Boulders, Roads, Bare Soil and Moss. 5) Shrub Cover, the proportion (in percentage) of the habitat covered by shrubs, scrubs and small scrubs. 6) Soil and Rock Cover, the proportion of the habitat covered by bare soil, gravels, rocks and boulders.

POLLINATOR COMMUNITY

In the springs of 2008 and 2009 we conducted surveys of pollinators in 21 of the 29 studied populations, 8 in Nature Parks and 13 in non protected area. Counts were done on several days throughout the flowering period from 11:00 am to 17:00 pm. Counts were made on groups of *E. popovii* plants of similar size across populations. We observed a total of 5169 flower visits (193 to 331 visits per population). Specimens (n=486) of most morphospecies were captured for later identification in the laboratory.

We estimated pollinator abundance as the number of flower visits observed per minute of observation. Pollinator richness (S_{obs}) was estimated as the number of pollinator species observed in the counts. To estimate pollinator diversity we used Hurlbert's PIE index. We also characterized pollinator identity. To do this, we established eight functional groups: 1) Large bees: bees with 10 mm or more in body length; 2) Small bees: bees with less than 10 mm in body length; 3) Butterflies: mostly Rhopalocera, all nectar collectors; 4) Beeflies: Long-tongued Bombyliidae; 5) Hover-flies: nectar- and pollen-collecting Syrphidae and short-tongued Bombyliidae; 6) Beetles: including species collecting nectar and/or pollen; 7) Ants: nectar-collecting worker ants 8) Others:

nectar-collecting small flies, wasps, bugs, and grasshoppers. Abundance of each functional group was estimated as the number of total flower visits observed pooling the insect species belonging to each functional group.

HERBIVORY DAMAGE

Main *E. popovii* herbivores in the area are domestic (cattle, sheep and goat) and wild (Spanish ibex: *Capra pyrenaica*) ungulates. These herbivores consume the flowering and fruiting stalks of the plants, damaging most fruits and destroying the seeds (Gómez 2005b). To estimate herbivory damage, we randomly marked 20 plants per population in the spring of 2009, in the 29 populations. Then, in October 2009, at the end of the reproductive season, we recovered these plants and recorded whether they were unconsumed or consumed (totally or partially). With this data we estimates percent damage in each population.

POPULATION VIABILITY

We estimated the potential viability of each population by means of four variables. 1) Population size, the number of reproductive *E. popovii* individuals. The number of reproductive individuals was found in spring of 2008 by counting all plants flowering per population. We did no consider seedlings for obtaining population size. 2) Population density, the number of reproductive *E. popovii* individuals per square meter. For this, we divided population size by population area. The latter estimate was calculated by Polygon Convex using a portable GPS. 3) Seeds per fruit, the number of seeds in each ripe fruit. 4) Seeds per plant, the number of ripe seeds per *E. popovii* plant. To measure these twovariables, in September of 2008 we collected 30 plants per population, and counted the fruits produced by each plant. Then, in the laboratory, ripe seeds were counted using a magnifying glass.

DATA ANALYSIS

The effects of protection on habitat characteristics, pollinator assemblage, herbivory damage and population viability, were analyzed with three different kinds of models (Appendix B). First of all, we conducted linear models using generalized least squares for all variables. Then, to account for spatial distribution, we included the geographic coordinates of each population and re-fitted the models to transform them in spatially explicit models. We first re-fitted the models with an exponential spatial correlation structure (exp(-r/d)), and then, with a Gaussian spatial correlation structure (exp(-(r/d)^2)). In both cases d denotes the range and r the distance between the observations. All analyses were performed using the package nlme in R (R Development Core Team 2008). We used the Gaussian spatial correlation because most AICs (Akaike Information Criterion, an estimate of the model suitability) of these models were lower than the AICs from Exponential models. We used sequential Bonferroni correction to get the significance of the different models, to control for possible table-wise type I errors produced by the high number of models. We also performed more complex linear models including Region as an additional independent variable. We compared the AIC of these models with the AIC values of the first models (only Protection as independent factor). When the AIC's of the more complex models were lower than the AIC's of the simple models, we concluded that Region was significantly affecting the dependent variables (Burnham and Anderson 2002). In these cases, we also calculated the p value of the factor Protection (shown in Appendix B).

RESULTS

HABITAT DIFFERENCES BETWEEN PROTECTED AND NON PROTECTED AREAS

E. popovii populations from protected areas were located at higher altitude than those inhabiting non protected areas (Fig. 2a) (Table 1). However, the altitude lost its significance when Region was included in the model (Appendix B). In addition, shrub cover was significantly higher in populations from protected areas (Fig. 2c) (Table 1). There were also significant differences between protected and non protected areas in the density of co-occurring flowering plants, but this difference was not significant after Bonferroni correlation (Fig. 2b) (Table 1) and not significant either in the models including Region (Appendix B). The remaining habitat variables (co-occurring flowering plant diversity, habitat diversity, bare soil cover and rock cover) showed no differences between protected and non protected areas (Table 1).

Variable	Gaussian		
	AIC	t	P
Altitude	341.25	2.81	0.009*
Co-occurring Flowering Plant Diversity	13.23	0.20	0.844
Co-occurring Flowering Plant Density	251.03	2.54	0.017
Habitat diversity	-48.04	0.40	0.691
Shrub Cover	-16.91	2.94	0.007*
Bare Soil and Rock Cover	-7.31	-0.65	0.520

Table 1. *Differences in habitat characteristics between protected and non protected areas. Significant values after Bonferroni correction are marked with an asterisk.*

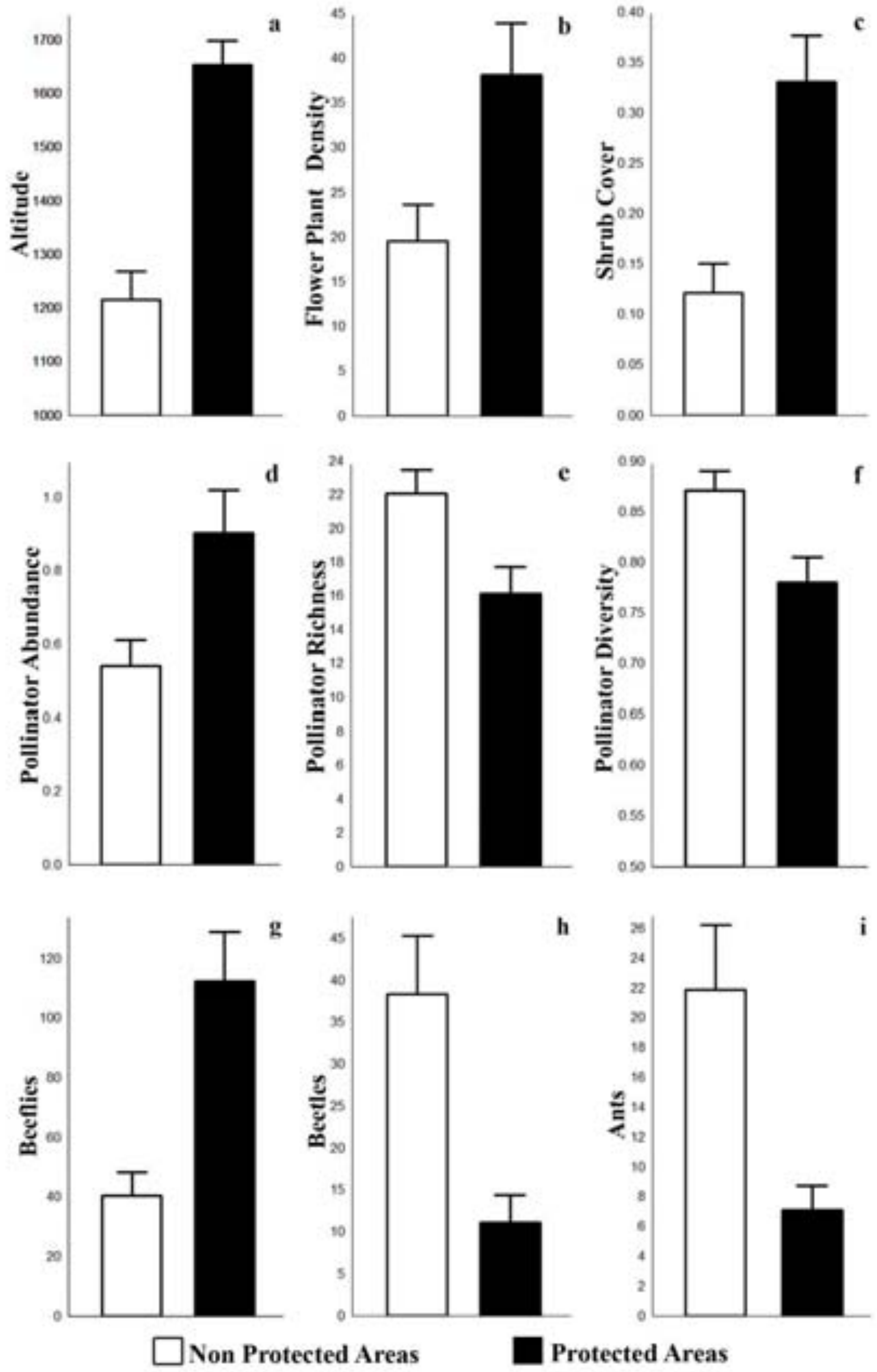

Figure 2. *Mean and Standard Error of habitat characteristics and pollinator assemblage in protected and non protected areas: a) altitude, meters above the sea level; b) co-occurring flowering plants density, flower per square meter; c) cover percentage of shrub; d) number of pollinator visits per minute of observation; e) number of pollinator species; f) Hurlbert's PIE diversity index of pollinators; g) beefly abundance; h) beetle abundance; and g) ant abundance. Only variables that were significant in the spatially-explicit models are shown.*

EFFECT OF PROTECTED AREAS ON POLLINATORS

We found significant differences between protected and non protected areas in the pollinator community of *E. popovii* (Table 2). Specifically, populations in protected areas showed higher flower visitation rates; that is, plants in protected areas received more visits per minute (Fig.2 d). In contrast, we observed lower richness and diversity of pollinator in populations from protected areas (Fig. 2 e, f). However, pollinator richness did not show differences between protected and non protected areas when Region was considered (Appendix B). There were also differences in pollinator composition. Beeflies were significantly more abundantin populations from protected areas (Fig.2 g). On the other hand, ants and beetles were more abundant in populations located outside protection areas (Fig. 2 h, i) (Table 2). The abundances of the large bees, small bees, butterflies, hoverflies and other pollinators were not related to protected areas (Table 2). Only beefly abundance was significant after Bonferroni correction, even when Region was included in the model (Appendix B).

Variable	Gaussian		
	AIC	t	P
Flower Visitation Rate	19.41	2.303	0.0327
Flower Visitation Richness	128.6	-2.587	0.0181
Flower Visitation Diversity	-32.7	-2.54	0.02
Large Bees	211.8	0.946	0.356
Small Bees	191.7	-1.366	0.1878
Butterflies	162.7	-0.386	0.7034
Beeflies	204.7	3.975	0.0008*
Hoverflies	138.9	-1.528	0.1428
Beetles	177.4	-2.18	0.0416
Ants	162.9	-2.251	0.0364
Others	173.1	0.583	0.5664

Table 2. *Differences in pollinator community between protected and non protected areas. Significant values after bonferroni correction are marked with an asterisk.*

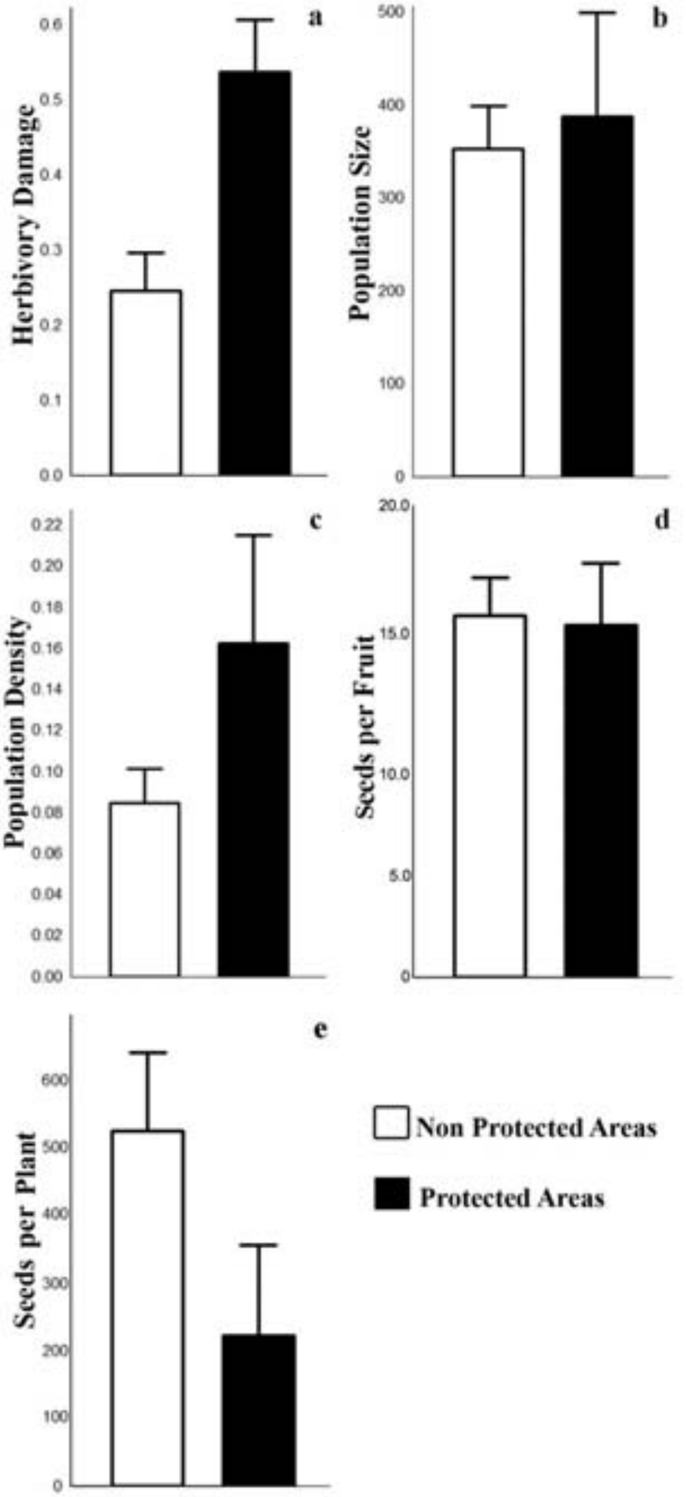

Figure 3. *Mean and Standard Error of herbivory damage and population characteristic variables in protected and non protected areas: a) percentage of damaged plants; b) number of total E. popovii reproductive individuals in each population; c) number of E. popovii reproductive individuals per square meter in each population; d) mean of number of seeds per fruit; and e) number of E. popovii seeds per square meter in each population.*

EFFECT OF PROTECTED AREAS ON HERBIVORE DAMAGE

Damage by ungulates was positively related to protection (AIC=1.297, t=2.485 and p=0.022). That is, *E. popovii* in protected areas was significantly more damaged than outside protected areas (Fig. 3 a). Herbivory outside protected areas averaged 24,61% and ranged from 0 to 60%. In populations inside protected areas herbivory rose to 53,75%, ranging from 15 to 90%.

POPULATION VIABILITY INSIDE AND OUTSIDE PROTECTED AREAS

We removed from our analyses one population (Population 30), since its protection status changed during the study period. So, we only used 28 populations to estimate population viability. Surprisingly, population size and density did not differ inside and outside protected areas (Table 3, Fig. 3 b, c). Similarly, seeds per fruit were similar inside and outside protected areas (Table 3d). However, number of seeds per plant was significantly higher in non protected areas (Table 3e), although significance vanished when Region was considered (Appendix B).

Variable	Gaussian		
	AIC	t	P
Population size	386.26	0.22	0.829
Population density	-16.11	1.34	0.192
Seed per fruit	165.56	-0.004	0.997
Seeds per plant	361.94	-2.77	0.010*

Table 3. *Differences in the estimates ofpopulation viability between protected and non protected areas. Significant values after Bonferroni correction are marked with an asterisk.*

DISCUSSION

Our results indicate that *E. popovii* populations differ in habitat characteristics depending on their protection status. In particular, populations in protected areas are located at higher altitudes than populations in non protected areas. This finding reflects the conservation strategy employed in the Iberian Peninsula and the Mediterranean Region in general. In this region, mountain areas are considered a conservation priority for two main reasons: they represent important biodiversity hotspots (Médail & Quezel 1999; Myers et al. 2000) and are exceptional observatories for evaluating the harmful consequences of global change (Malcom et al. 2006). Nevertheless, this result should be considered with caution, because altitude was not significant when Region was considered. In general, although our populations were located in discrete regions and populations from a given region used to belong to the same protection category (Fig. 1), we found that when including this variable in the statistical analyses the outcome remains similar in most cases (Appendix B). This finding suggests that the effect of protection did affect *E. popovii* populations irrespective of the specific region they were located.

We also found that protected populations were located in areas with higher shrub plant cover than non protected populations. The difference in altitude between protected and non protected areas may be a first reason accounting for this difference in habitat. However, we think that in our study system the effect of altitude is negligible. The altitude of our populations ranges between 1000 and 2000 meters. The habitat along this altitude range is composed of evergreen *Quercus-Pinus* mixed forest in most Mediterranean mountains from the Iberian Peninsula (Blondel et al. 2010). In contrast, most present-day differences in habitat in our study site is caused by human activities rather than differences in altitude. For this reason, we believe that the difference in shrub cover that we found may be a consequence of the protection practice itself, since scrublands and woodlands usually colonize many old fields within protected areas due to the abandonment of agricultural practices (Cramer et al. 2008). In southern Iberian peninsula, a process of secondary succession usually follows protection of a given area, where shrubs colonize former meadows and grasslands. Some *E. popovii* populations are located in this type of habitats. The consequences of this ecological process for the conservation and persistance of endangered organisms inhabiting open sites, including herbs, is still unclear (e.g., Balmer & Erhardt 2000; Steffan-Dewenter & Leschke 2003; Cremene et al. 2005). For *E. popovii*, higher woody plant cover have been related with low levels of pollen limitation (Fernández et al. 2012), which could increase seed production and favor population persistence.

Two fundamental reasons may justify the study of the effect of protected areas on pollinators. The first one is related to the direct interest in conservation of flower visitors, mostly taking into account that they are declining in many parts around the world (Kearns et al. 1998; Kremen & Ricketts 2000; Biesmeijer et al. 2006; Cameron et al. 2011). A second reason is related to the role that these organisms play for the reproduction and therefore conservation of many endangered plants (Waser 1978; Motten et al. 1981; Snow 1982; Steffan-Dewenter et al. 2001; Waites & Agren 2004). Our study also found higher pollinator abundance in protected areas than in non protected ones (Table 2). These results are similar to results obtained by Kremen et al. (2004), who found a strong relationship between non-altered habitats and pollination services. Similarly, negative correlation between pollinator visitation rate and distance to non-altered habitats has been reported by Carvalheiro et al. (2010). In contrast, pollinator richness and diversity in our study was higher in populations located outside protected areas. Since protected and non protected areas are located at different altitudes, this difference in pollinator fauna may be an indirect consequence of the difference in altitude. However, in many Mediterranean mountains, including the Sierra Nevada, the diversity and abundance of insects gradually increases from 1000 m a.s.l. upward until reaching a peak approximately at 2500 m (González-Megías et al. 2008 and references there in). For this reason, we should have found a higher pollinator diversity in protected areas, that were located at higher altitude, than in non protected populations. In addition, habitat diversity did not vary either between protected and non protected areas. For all these reasons, we believe that the difference in pollinator fauna may be explained by the more heterogeneous habitat composition of non protected areas (Quintero et al. 2010), which would favor a more diverse array of pollinators.

From the point of view of plant reproduction, pollinator identity may be more important than pollinator diversity, because different pollinator functional groups usually have different flower-handling behaviors and show important differences in the amount and quality of pollen deposited per visit (Fenster & Dudash 2001; Cosacov et al. 2008; Gómez et al. 2010). Our study showed variation in pollinator composition in populations from protected and non protected areas (Fig. 2g). The most important result in this sense was that beeflies were more abundant in protected areas. This may have important implications for plant conservation, because beeflies are efficient *Erysimum* pollinators (Gómez et al. 2009; Fernández et al. 2012). In fact, we have found that an increase in beefly visitation rate is associated with a decrease in *E. popovii* pollen limitation intensity (Fernández et al. 2012). On the other hand, low-efficiency pollinators, such as ants and beetles, were more abundant in non protected areas (Fig. 2g). These insects are usually generalist flower visitors, and usually inhabit areas with high levels of disturbance. Bees showed no preference for protected or non protected areas. This may be explained based on studies showing that bee species richness is highest in habitats with intermediate levels of human disturbance (Winfree et al. 2007). In addition, it has been proposed that bees are adapted to altered habitat (Carvell 2002; Eltz et al. 2002; Klein et al. 2002). Altogether, our findings suggest that protected areas would provide a better pollination service, since pollinator assemblages in these areas are composed of more efficient pollinators (i. e. beeflies).

We found higher herbivory damage in protected areas than in non protected ones. Most of the damage was produced by domestic animals: Cattle, sheep and goats (field observations), which usually have a higher impact on plants than wild animals (Albon et al. 2007). This high abundance of domestic livestock is a consequence of the encouragement and promotion of traditional uses in the protected areas harboring *E. popovii* populations. It is widely accepted that low or intermediate level of herbivory may benefit plant reproduction, enhance plant diversity and help to protect the populations of some plants (Olff & Ritchie 1998). The landscape heterogeneity and diversity produced by the long-standing herbivory would have historically enhanced the biodiversity found now in many Mediterranean ecosystems (Verdú et al. 2000). However, when the abundance of herbivores is too high, their effect on plant community turns negative, particularly in the Mediterranean mountain areas with several rare or endemic species (González-Megías et al. 2004; Dostálek et al. 2008, Ancheta & Heard 2011). In this respect, many Mediterranean mountains are overgrazed due to the high abundance of both domestic and wild ungulates (Zamora et al. 1999). As a consequence, it has been shown that ungulates collapse the reproduction and recruitment of many Mediterranean woody and herbaceous plants (Gómez & Zamora 2000; Zamora et al. 2001; Focardi & Tinelli 2005; Gómez & Hódar 2008; DeSoto et al. 2010; Gabay et al. 2011). In our case, the observed high level of damage may constrain recruitment of *E. popovii* populations, as has been shown for other *Erysimum* species (Gómez 2005a).

The most striking outcome of our study is related to the fact that *E. popovii* populations inhabiting protected areas do not benefit of such protection. All protected *E. popovii* populations are located in areas belonging to the category V of the UICN. This category was implemented in 1994 to resolve the conflict between economic development and conservation of habitat and endangered species (IUCN 1994). According to the Task Force on Protected Areas Categories, the main objectives of this category are: 1) "To maintain harmonious interaction of nature and culture through the protection of landscape and/or seascape and the continuation of traditional land uses, building practices and social and cultural manifestations"; 2) "To support lifestyles and economic activities which are in harmony with nature and the preservation of the social and cultural fabric of the communities concerned"; and 3) "To maintain the diversity of landscapes and habitat, and of associated species and ecosystems" (Phillip and Brown 2007). *Erysimum popovii* protected populations did not contain more individuals per population and per square meter or produced more seeds than non protected populations. We believe that this happens because the overgrazing occurring in these areas counteracts the potential benefits provided by pollinators, not only by consuming plants and seeds, but also by trampling seedlings and juveniles (Gómez 2005a). The negative effect of herbivory is offsetting the positive effects of pollinator visitation in protected areas. As a consequence, the viability of *E. popovii* population seems to be similar inside and outside protected areas.

CONCLUSIONS

Our results suggest that the balance between human uses (mostly grazing in our case) and conservation is insufficient for some plants species such as *E. popovii*. We need to be cautious with our conclusions, because we have just studied one single plant species inhabiting a peculiar environment, the Mediterranean mountains. In this type of environment, grazing has helped to conform the highly diverse present-day landscape and has contributed to preserve many of the plants we enjoy now. However, we believe that this positive effects may turn negative for some plant species when herbivory pressure become too intense. Consequently, we believe that, if a goal of conservation practitioners is to design useful protected areas, they should reduce the intensity of certain harmful traditional uses. In particular, in protected areas of the Mediterranean region it would be necessary to diminish grazing intensity by reducing sheepherding authorizations and establishing permanent grazing exclusion zones. As suggested by Locke & Dearden (2005), many Nature Parks should not be considered protected areas. These areas should be better reclassified as sustainable development areas, establishing small Natural Reserves within them, corresponding to Category IV of the World Conservation Union classification (IUCN 1994). This partial conversion of the protected areas under the category V to category IV in the Mediterranean region would surely help to elude conflicts between human development and nature conservation.This is especially important in the Mediterranean region, where Category V represents 62% of the total protected surface. Such an approach would generate a heterogeneous landscape with some true protected areas, Natural Reserves, within a matrix of sustainable development areas, Nature Parks. Since it is crucial not to confront conservation policies with the interests of local residents, acting in this way could allow to combine both traditional uses and nature. To delimit special sites with strict protection rules inside existing protection areas could ensure the conservation of vulnerable plant species (Vellak et al. 2010). Further similar studies are necessary to understand if our outcomes may be relevant for other rare plants inhabiting human-shaped enviroments.

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APPENDICES

Appendix A. *Location and characteristics of the 29 E. popovii populations studied.*

Population	Region	Coordinates (UTM)	Altitude	Population Size	Population Density	EUNIC habitat Classification	Dominant Plant Species	Protection
Ep01	Mencal, Granada	30S 0487836 4155063	982	600	0.015	G4.B	*Pinus halepensis; Juniperus phoenicea*	No
Ep02	S. Arana, Granada	30S 0451977 4128858	1255	340	0.011	G2.12411	*Stipa tenacissima; Quercus rotundifolia*	No
Ep03	S. Nevada, Granada	30S 0456751 4109531	1596	280	0.037	F3.11	*Prunus prostrata; Santolina rosmarinifolia; Lavandula lanata*	P.N Sierra Nevada
Ep04	S. Nevada, Granada	30S 0467701 4118300	1503	300	0.073	F6.73	*Berberis hispanica; Genista scorpius; Thymus mastichina*	P.N Sierra Nevada
Ep05	S. Jabalcuz, Jaen	30S 0425545 4177799	1321	420	0.197	G4.B	*Pinus halepensis; Thymus orospedanus; Erinacea anthyllis*	No
Ep07	S Mágina, Jaen	30S 0453888 4176684	2000	500	0.135	F3.164	*Juniperus sabina*	P.N. Sierra Mágina
Ep08	S. Jabalcuz, Jaen	30S 0425519 4177931	1324	400	0.173	G4.B	*Pinus halepensis; Genista cinerea; Rhamnus saxatilis*	No
Ep09	La Peza, Granada	30S 0472834 4119460	1693	130	0.085	F6.73	*Berberis hispanica; Cytisus scoparius; Thymus mastichina*	P.N Sierra Nevada
Ep10	S. Jabalcuz, Jaen	30S 0424122 4177754	1060	450	0.064	F3.11	*Crataegus sp.; Genista cinerea*	No
Ep11	S. Horconera, Cordoba	30S 0386985 4137896	1282	170	0.018	G2.12411	*Stipa tenacissima; Quercus rotundifolia*	P.N. Subbéticas
Ep13	S. Arana, Granada	30S 0454337 4131901	1738	370	0.087	F6.73	*Erinacea anthyllis; Hormathophylla spinosa*	No
Ep14	S. Jabalcuz, Jaen	30S 0426894 4180210	970	120	0.025	E1.513	*Brachypodium retusum; Phlomis purpurea*	No
Ep15	S. Jabalcuz, Jaen	30S 0425148 4179387	1080	650	0.062	E1.513	*Phlomis purpurea; Brachypodium sp.*	No
Ep16	S. Jabalcuz, Jaen	30S 0425152 4179487	1060	150	0.038	G3.74	*Pinus halepensis*	No
Ep17	Mencal, Granada	30S 0488073 4154212	1030	70	0.065	E1.41	*Stipa tenacissima; Juniperus oxycedrus; Thymus orospedanus*	No
Ep18	Mencal, Granada	30s 0487810 4154495	1063	50	0.034	G3.74	*Pinus halepensis*	No
Ep19	Mencal, Granada	30S 0483826 4151106	1188	420	0.246	G2.12411	*Quercus rotundifolia; Rosmarinus officinalis*	No
Ep20	S. Arana, Granada	30S 0451048 4130531	1270	350	0.069	E1.41	*Stipa tenacissima; Brachipodium sp.*	No
Ep21	S. Arana, Granada	30S 0452839 4131133	1498	280	0.113	F6.73	*Erinacea anthyllis: Lavandula sp.*	No
Ep22	S. Arana, Granada	30S 0453676 4128236	1380	620	0.069	F5.58	*Lavandula sp; Ulex parviflorus*	No
Ep23	La Peza, Granada	30S 0472949 4119278	1720	750	0.556	F6.73	*Cytisus scoparius; Genista versicolor*	P.N Sierra Nevada
Ep24	La Peza, Granada	30S 0472575 4119078	1746	60	0.011	F6.73	*Cytisus scoparius; Santolina rosmarinifolia*	P.N Sierra Nevada
Ep25	La Peza, Granada	30S 0473086 4118783	1757	370	0.091	F5.56	*Genista versicolor*	P.N Sierra Nevada
Ep26	La Peza, Granada	30S 0471754 4118764	1757	220	0.054	G2.12411	*Erinacea anthyllis; Festuca sp.*	P.N Sierra Nevada
Ep27	S. Nevada, Granada	30S 0457430 4109448	1486	340	0.176	E1.513	unidentified herb	P.N Sierra Nevada
Ep28	S Mágina, Jaen	30S 0455789 4176686	1661	1650	0.193	G2.12411	*Erinacea anthyllis*	P.N. Sierra Mágina
Ep29	S Mágina, Jaen	30S 0456670 417576	1525	19	0.633	G2.12411	*Erinacea anthyllis; Quercus rotundifolia*	P.N. Sierra Mágina
Ep30	S Mágina, Jaen	30S 0453436 4176812	1898	2050	0.133	E1.513	*Berberis hispanica;* unidentified herb	P.N. Sierra Mágina
Ep31	S. Nevada, Granada	30S 0467790 4118261	1512	260	0.048	F6.73	*Stipa tenacissima; Thymus sp.*	P.N Sierra Nevada

Appendix B. *Comparison between AIC`s values of two kind of spatially explicit models per each dependent variable studied, first using Protection as independent variables and then including Protection and Region as independent variables. When both models were significantly different (AIC of the latter model lower than AIC of the former model), we also show the p value of Protection.*

Variable	Models with Protection	Models with Protection + Region	
	AIC	AIC	p value of protection
Altitude	341.2492	292.5456	0.2684
Co-occurring Flowering Plant Diversity	13.23224	26.63279	
Co-occurring Flowering Plant Density	251.0284	225.7362	0.6609
Habitat diversity	-48.03633	-30.05383	
Shrub Cover	-16.90945	-6.024091	
Bare Soil and Rock Cover	-7.308538	7.613141	
Herbivory	1.297225	4.038256	
Flower Visitation Rate	19.41073	24.77746	
Flower Visitation Richness	128.6134	118.0443	0.4278
Flower Visitation Diversity	-32.65265	-17.55691	
Large Bees	211.8028	187.6389	0.1376
Small Bees	191.6721	172.0207	0.2167
Butterflies	162.6757	149.2305	
Beeflies	204.6971	207.6174	0.0041
Hoverflies	138.8725	122.3333	
Beetles	177.3527	156.0447	0.0116
Ants	162.9288	162.7451	
Others	173.1434	154.2251	0.6068
Population size	386.2643	338.8302	0.6260
Population density	-16.11298	-2.890448	
Seed per fruit	165.5626	201.204	
Seeds per plant	361.9413	310.6702	0.8991

DISCUSIÓN GENERAL

Erysimum popovii es polinizada por 165 especies de muy diverso tamaño, morfología y comportamiento y pertenecientes a diversos grupos taxonómicos. Esta alta riqueza específica en el conjunto de polinizadores nos indica que se trata de una especie generalista en cuando a sus polinizadores. Sin embargo, a pesar de esta gran diversidad en el espectro de polinizadores, observamos cierto grado de estructuración, existiendo una mayor abundancia de abejas grandes y bombílidos (Figura 1). Este pequeño grado de especialización dentro del generalismo podría suponer una ventaja reproductiva para la especie, ya que un exceso de diversidad en el espectro de polinizadores puede tener consecuencias negativas para la eficacia reproductiva. En este sentido Sahli y Conner (2006) encontraron que un incremento en la diversidad de polinizadores, conlleva un aumento de especies de polinizadores con bajas tasas de visitas, lo que podría incrementar la deposición de polen heteroespecífico, dificultando la reproducción de la planta. En investigaciones realizadas con *E. mediohispanicum* se encontró un óptimo de diversidad de polinizadores (Gómez et al., 2007), por encima y por debajo del cual disminuye el éxito reproductivo de las plantas. Sin embargo, en el caso de *Erysimum popovii* no encontramos ninguna relación entre la diversidad de los polinizadores presentes en cada población y las variables de reproducción estudiadas: limitación por polen (Capítulo 2), producción de frutos, producción de semillas, germinación, supervivencia de las plántulas, peso de las semillas, velocidad de germinación ni fecundidad (Capítulo 1).

En cambio, sí encontramos una correlación entre abundancia de bombílidos y altas tasas de emergencia de plántulas (Capítulo 1). Esto puede deberse al comportamiento de pecoreo de estos polinizadores, ya que realizan numerosas visitas cambiando continuamente de planta, lo que podría favorecer el entrecruzamiento (Gómez et al. 2010, Capítulo 2 y 3). Esta deposición alógama de polen puede disminuir la tasa de abortos y mejorar las semillas desde un punto de vista genético, produciendo plántulas más vigorosas y mejorando por tanto su tasa de emergencia (Vaughton and Ramsey 2010). Esta idea está reforzada por los resultados de las investigaciones del Capítulo 2, donde la abundancia de bombílidos estuvo asociada con una baja intensidad en limitación por polen (Capítulo 2). La alta eficacia de los bombílidos como polinizadores ha sido observado en otras especies del género *Erysimum* (Gómez et al. 2009, 2010) y en otros géneros de plantas (Motten et al. 1981; Johnson and Dafni 1998; Kastinger and Weber 2001; Anderson et al. 2005; Koopman and Ayers 2005).

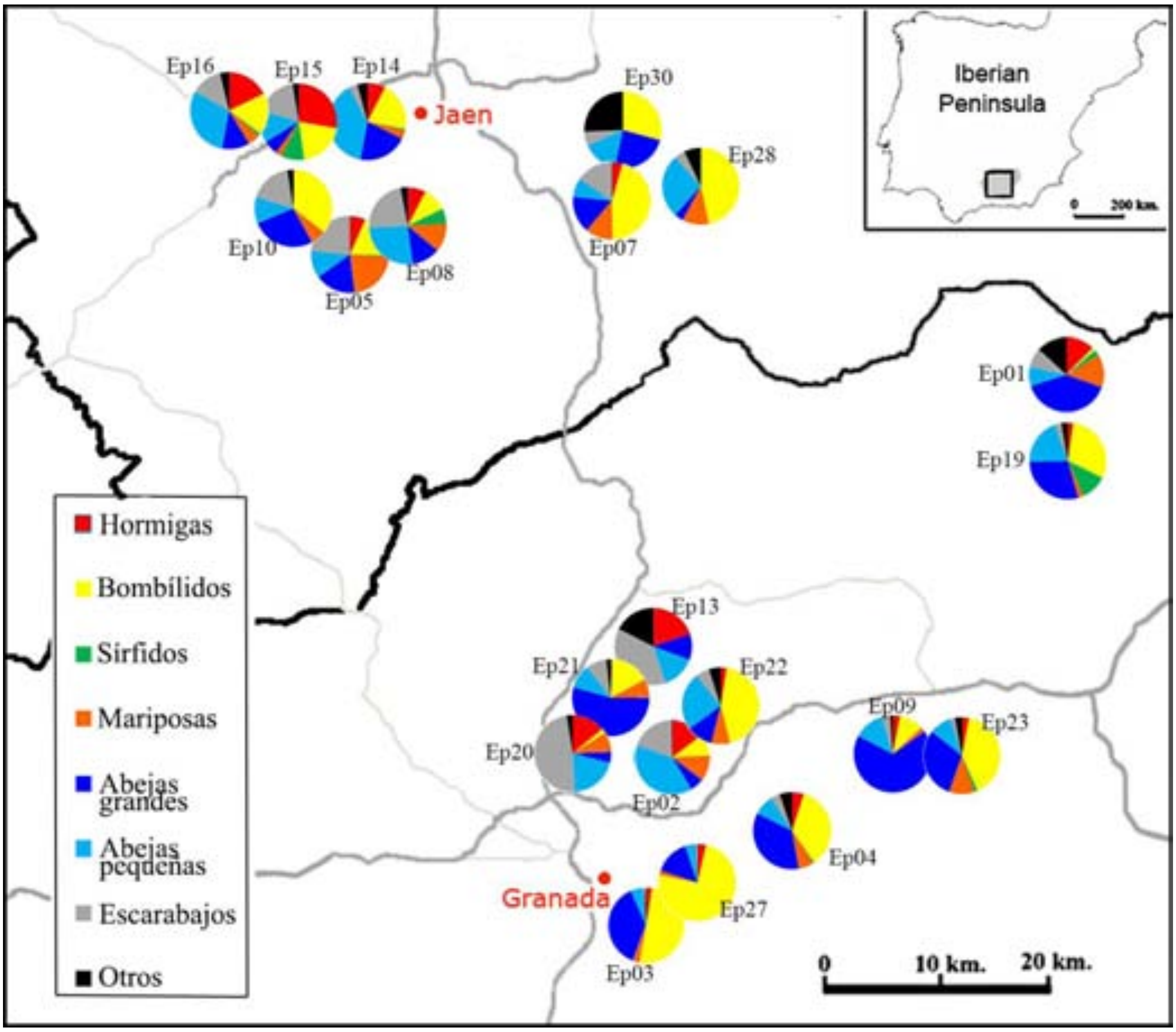

***Figura 1**: Distribución espacial de los polinizadores de E. popovii agrupados en grupos funcionales en las 21 poblaciones en las que se hicieron suficientes censos de polinizadores.*

Por tanto es probable que los bombílidos sean un grupo de polinizadores altamente eficiente como vectores polínicos de *Erysimum popovii*. Esto conlleva que, en las poblaciones 03, 04, 07, 10, 22, 23, 27, 28 y 30, donde hay una predominancia de bombílidos (Tabla 1), los individuos de *E. popovii* pueden tener ciertas ventajas reproductivas, con respecto a poblaciones donde los polinizadores predominantes son otros. Además es importante tener en cuenta que la abundancia de bombílidos es mayor en poblaciones con una alta abundancia de matorral y baja precipitación, según el análisis de correspondencias canónicas (Figura 2). Este análisis también mostró una correspondencia entre la densidad de individuos de *E. popovii* y la presencia de bombílidos.

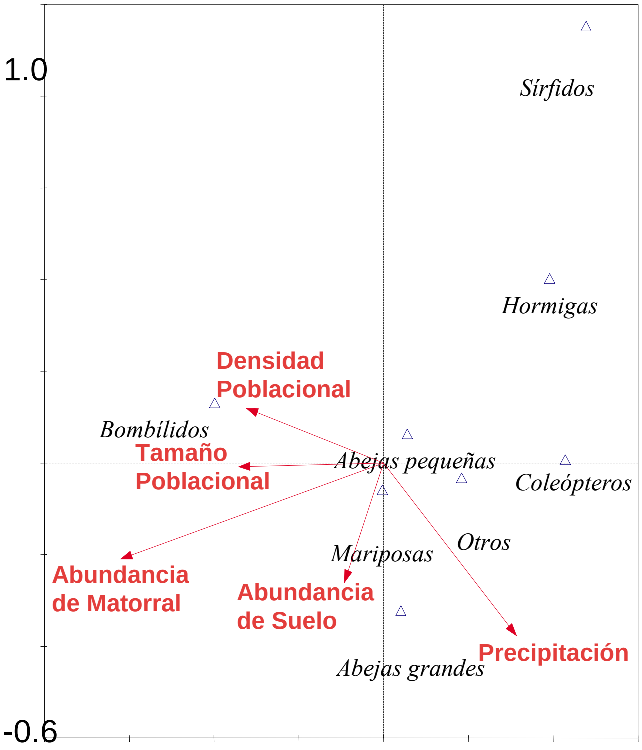

Figura 3: *Representación gráfica de los resultados del CCA, donde se observa la relación entre las variables seleccionadas y los grupos funcionales de polinizadores. Nota: solo se representan las variables que resultaron significativas en el test de Montecarlo (p<0.05, nº permutaciones=999).*

Dada la importancia de los bombílidos para la reproducción de *E. popovii* (Capítulo 1, 2 y 3) se hace necesario determinar los factores que pueden afectar a la presencia de bombílidos, sobre todos aquellos que han mostrado un efecto negativo sobre la abundancia de los polinizadores a largo plazo como el cambio climático (Memmott et al. 2007, UNEP 2010), insecticidas (Desneux et al. 2007, Regiane et al. 2013, Scholer & Krischik 2014), etc.

Por otra parte, la abundancia de polinizadores de baja calidad (hormigas, escarabajos, sírfidos y algunos grupos minoritarios) afectó negativamente a la reproducción de *Erysimum popovii*. La presencia de este tipo de polinizadores se correlacionó con bajos niveles de fructificación y una baja velocidad de germinación (Capítulo 1). Este resultado puede estar relacionado con la posible competencia existente entre *Erysimum popovii* y otras especies de plantas que florecen al mismo tiempo. En el Capítulo 1 se observó que en las poblaciones con una alta abundancia de plantas que florecen al mismo tiempo que *E. popovii* las tasas de visita de polinizadores de baja calidad eran mayores. Lo que nos sugiere que *E. popovii* no es muy atractiva para los polinizadores, en aquellas poblaciones donde la comunidad floral es abundante. La competencia sobre la atracción de polinizadores puede afectar de forma negativa a los individuos de *E. popovii*, ya que la disponibilidad de polinizadores es uno de los factores que más determinan la limitación de polen en las plantas (Lay et al. 2011). De hecho, la densidad de especies florales acompañantes afectó de forma negativa a la fructificación, con una menor proporción de flores que producían frutos en las poblaciones donde la densidad de otras plantas que florecen al mismo tiempo que *E. popovii* es mayor, como las poblaciones 13 y 22. En estas dos poblaciones es donde la densidad de especies florales acompañantes es mayor y su fructificación fue de un 50% aproximadamente. Dado que la mayor parte de las especies que florecen al mismo tiempo que *Erysimum popovii* son herbáceas o arbustos, podríamos concluir que ambos grupos afectan de forma negativa por competencia a la atracción de polinizadores durante el periodo de floración.

Poblaciones	Número de individuos	Altitud	Abundancia de Polinizadores (visitas/hora)	S_{obs} Polinizadores	Principales Polinizadores	Semillas por individuo	Plántulas por individuo	Fecundidad (tasa de reclutamiento)	Tipo de limitación por polen	Limitación de Reclutamiento	Herbivoría (% plantas dañadas)	Área Protegida
01	600	982	30.6	21	Abejas	476.73	239.668	0.00	Si	Micrositios	10%	No
02	340	1255	27.0	22	Abejas	279.45	14.874	-	-	-	45%	No
03	280	1596	88.8	13	Bombi. y Abej.	563.06	151.183	1.69	No	Semillas	45%	Si
04	300	1503	55.8	24	Bombi. y Abej.	245.36	60.242	9.38	-	Micrositios	50%	Si
05	420	1321	19.8	22	Sin preferencias	578.68	39.583	0.27	Si	Semillas	0%	No
07	500	2000	26.4	13	Bombílidos	277.63	5.308	0.23	-	Micrositios	6%	Si
08	400	1324	22.2	20	Escar. y Abej.	296.73	13.665	0.71	Si	Sem. y Disper.	5%	No
09	130	1693	66.0	13	Abejas	400.45	12.728	2.22	No	Micrositios	60%	Si
10	450	1060	34.8	23	Bombi. y Abej.	649.46	48.027	1.33	Si	Sem. y Disper.	10%	No
13	370	1738	43.2	16	Escarabajos	271.54	-	-	Si	Dispersión	60%	No
14	120	970	55.2	32	Abejas	956.22	240.143	1.78	-	Semillas	15%	No
15	650	1080	25.8	28	Abejas	1055.6	130.685	1.24	No	Semillas	25%	No
16	150	1060	14.4	18	Hormigas	677.04	152.603	6.39	Si	Micrositios	15%	No
19	420	1188	24.0	29	Abejas	412.77	36.217	0.13	Si	Semillas	10%	No
20	350	1270	17.4	25	Escarabajos	378.93	66.545	2.49	Si	Sem. y Disper	50%	No
21	280	1498	72.0	15	Abejas	395.38	43.723	1.02	Si	Dispersión	35%	No
22	620	1380	33.6	16	Bombílidos	220.21	24.223	-	Si	-	40%	No
23	750	1720	40.8	20	Bombi. y Abej.	484.23	46.864	0.24	-	Micrositios	5%	Si
27	340	1486	64.8	11	Bombílidos	406.42	60.599	0.13	-	Dispersión	60%	Si
28	1650	1661	30.6	21	Bombílidos	263.61	26.142	0.46	-	Dispersión	15%	Si
30	2050	1898	60.0	14	Bombi. y Abej.	128.64	19.940	0.60	-	Dispersión	90%	Si

Tabla 1. *Datos generales de las 21 poblaciones de E. popovii estudiadas en la tesis.*

Sin embargo, estas interacciones interespecíficas son distintas en otros estadios de la planta. Por ejemplo durante el paso de semilla a adulto, los matorrales pueden ejercer un efecto de facilitación sobre *E. popovii*, actuando como plantas nodrizas, al protegerlos de la herbivoría y de las inclemencias meteorológicas (Capítulo 3), algo de especial importancia en el clima mediterráneo, dada la falta de precipitación durante el período estival (Pugnaire et al. 2001; Rey et al. 2004; Gómez-Aparicio et al. 2004). Sin embargo, las especies herbáceas pueden competir con *E. popovii* por la disponibilidad de micrositios para germinar y el desarrollo de las plantas adultas. De hecho, en el capítulo 3 observamos como en aquellas poblaciones con una mayor densidad de especies herbáceas, se producía una limitación por micrositios, sugiriendo una posible competencia interespecífica (Schoener 1983, Brandt & Seabloom 2012).

Las poblaciones de *Erysimum popovii* están limitadas por diversos factores no excluyentes. El reclutamiento de la especie puede estar limitado por la producción de semillas, por la dispersión de las semillas y/o la disponibilidad de micrositios. Siendo las limitaciones por producción y dispersión de las semillas las más comunes (Capítulo 3). En este sentido, la depredación de las semillas por parte de herbívoros puede ser un factor determinante sobre el éxito reproductivo de la planta y por tanto sobre su conservación como se ha demostrado en el Capítulo 4, así como en otras muchas investigaciones con diversas especies vegetales (Crawley 1989; Vargas et al. 2002; Gómez et al. 2003; Gómez 2005; Albon et al., 2007; Baraza et al. 2007; Uytvanck et al. 2010). Sin embargo, los herbívoros pueden tener un efecto positivo sobre la capacidad de dispersión de la especie mediante el transporte accidental de semillas, así como un posible efecto positivo al reducir la densidad de las especies vegetales más abundantes favoreciendo la disponibilidad de micrositios (Grime 1973). De esta forma, cierto grado de herbivoría podría disminuir la limitación por dispersión o la limitación por micrositios en condiciones muy determinadas.

Es importante tener en cuenta que *Erysimum popovii* es una especie catalogada como “Casi Amenazada” en la lista Roja de la flora vascular de

Andalucía (Cabezudo et al. 2005). Por tanto los análisis sobre la demografía de la especie y de las variables que le afectan, son de vital importancia para su conservación (Capítulo 4). En este sentido, es fundamental conocer la evolución de los tamaños poblacionales para establecer la Población Mínima Viable (PMV). La PMV es el tamaño de población más pequeño de una determinada especie que, manteniéndose aislada de otras poblaciones, tiene un 99% de probabilidad de persistir durante al menos mil años (Shaffer 1981). Para ello realizamos proyecciones demográficas para cada una de las poblaciones, teniendo en cuenta el tamaño poblacional y las probabilidades de transición entre estadíos analizados en el Capítulo 1. Para este análisis no se consideró la aleatoriedad demográfica, ambiental o genética que pueda sufrir cada población, sino que se estudió la progresión del tamaño poblacional de acuerdo a las condiciones actuales y considerando constante el éxito reproductivo. Para dicho análisis empleamos el software Populus (Alstad et al. 2005).

Según nuestros análisis, la mayoría de las poblaciones de *E. popovii* podrían extinguirse en menos de 500 generaciones, es decir, 1000 años (tabla 2). El tamaño de Población Mínima Viable varía mucho, ya que depende de los datos de reproducción y de las probabilidades de transición entre estadios en cada una de las poblaciones, y por tanto de las condiciones ambientales. Aun así, como promedio, la población mínima viable de *E. popovii* podría situarse alrededor de los 500 individuos. En poblaciones con condiciones subóptimas el tamaño poblacional debería ser mucho mayor para poder asegurar su mantenimiento a largo plazo. Estas poblaciones están condicionadas sobre todo por la baja probabilidad de transición de plántula a adulto, siendo éste el momento más vulnerable del ciclo vital y, en ocasiones, por la limitación en la producción de semillas.

Si queremos usar estos datos como una estima real de la evolución de la especie, tendríamos que tener en cuenta que están calculados al alza, puesto que no se han incorporado datos procedentes de un posible banco de semillas, al no tener constancia de la existencia de estadios durmientes. Además, no se ha incluido es estos análisis el comportamiento de las poblaciones de *Erysimum popovi* como un sistema metapoblacional (Menges, 1990; Bonet et al. 2005; Rozenfeld et al. 2008), por lo que la aportación de individuos o de material genético procedente de otras poblaciones y la existencia de hábitats susceptibles de ser colonizados por la planta pueden llegar a ser bastante importantes para asegurar la viabilidad poblacional de la especie. En este sentido, una mejora de la conectividad de las poblaciones conllevaría una mayor probabilidad de persistencia local y regional, un aumento de su estabilidad y resiliencia frente a perturbaciones y de su capacidad de recolonización tras posibles extinciones locales (Crooks & Sanjayan, 2006 in: Saura et al. 2011)

Población	Tamaño poblacional	Viabilidad demográfica (nº generaciones)
01	600	2
03	280	454
04	**300**	**>1000**
05	420	269
07	500	373
08	400	360
09	**130**	**594**
10	450	421
14	120	363
15	650	356
16	**150**	**994**
19	420	307
20	**350**	**614**
21	280	394
23	750	271
27	340	238
28	1650	319
30	2050	397

Tabla 2. *Análisis de la viabilidad demográfica por población. Se muestra el número de la población, el tamaño poblacional (número de individuos) y el número de generaciones que tardaría la población en extinguirse, según la tasa de fecundidad y las probabilidades de transición entre estadios vitales calculadas en el Capítulo 1. En negrita se muestran las poblaciones que estarían por encima de la PMV.*

Para llevar a cabo un análisis de la conectividad de las poblaciones de *E. popovii*, determinamos el área de cada población y la distancia mínima entre los bordes de cada par de poblaciones mediante la extensión CONEFOR Inputs para ArcGIS. El cálculo de la probabilidad de dispersión directa entre cada par de poblaciones se hizo a partir de la distancia media de dispersión del polen mediante polinizadores (estimada en 500 m, de acuerdo a Gathman & Tscharntke, 2002; Steffan-Dewenter et al., 2002) mediante el programa informático CONEFOR SENSINODE 2.6 (Saura & Torné 2009), para estos análisis utilizamos los datos de 29 poblaciones. Según estos análisis el área de distribución de *E. popovii* se organiza en 23 componentes, es decir, tenemos 23 grupos de teselas que siguen una estructura de metapoblaciones; las poblaciones pertenecientes al mismo componente están conectadas entre sí en mayor o menor grado, tan solo la población Ep11, en base a las poblaciones estudiadas, se encuentra totalmente aislada del resto de poblaciones, lo que coincide con las características observadas en la población, tratándose de una población con una muy baja reproducción (observaciones en el campo) y posiblemente depauperada genéticamente. Calculamos el Índice de la Probabilidad de Conectividad (PC) para las poblaciones estudiadas, este índice se define como la probabilidad de que dos organismos situados al azar queden situados en zonas de hábitat que estén interconectadas, es decir, que sean capaces de encontrarse dado el mosaico de teselas y las conexiones existentes entre ellas (Saura y Pascual-Hortal, 2007). El índice PC para *E. popovii* es de 3.8, es decir existe un 3.8% de probabilidad de que dos puntos situados al azar en el área de distribución de *E. popovii* se puedan conectar entre sí.

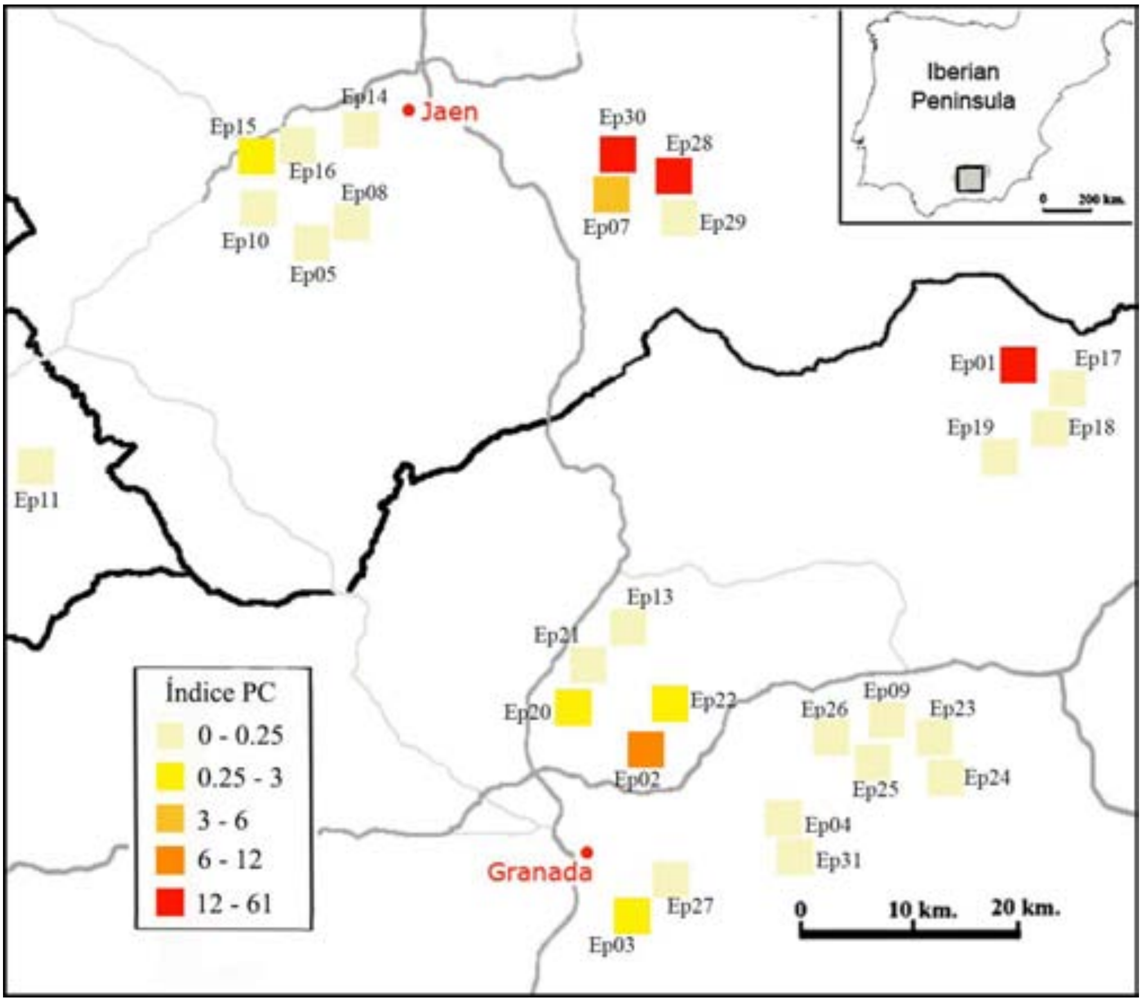

Figura 3*: Representación espacial del índice de conectividad de las 29 poblaciones de E. popovii poblaciones estudiadas.*

Los bajos valores calculados del Índice PC nos indican que *Erysimum popovii* es una especie con una pobre conectividad entre poblaciones. Esta conectividad depende de la poca dispersión que alcanza la lluvia de semillas, pero sobre todo de los polinizadores. En los análisis de conectividad se observó que uno de los factores más determinantes para la conectividad entre las poblaciones es el tamaño de las poblaciones incluidas en las teselas, así como su capacidad de reproducción. Por lo tanto, contar con teselas grandes y de buena calidad, es más importante para *E. popovii* que establecer vías de conexión entre las distintas poblaciones. Las poblaciones 1, 2, 28 y 30 son las que más contribuyen a la conexión entre diferentes teselas, especialmente la 28 y la 30 que además, podrían estar actuando como fuentes, por lo que éstas serían las áreas prioritarias de cara a su conservación.

RECOMENDACIONES PARA LA GESTIÓN Y CONSERVACIÓN

Una de las medidas más importantes para garantizar la conservación de *E. popovii* a largo plazo es el establecimiento de zonas de exclusión de herbivoría, al ser la presión de herbivoría un factor determinante sobra la conservación de las poblaciones (Capítulo 4). De esta forma se podría garantizar la supervivencia de la especie estableciendo zonas fuente de propágulos que favorezcan la dinámica metapoblacional de la especie, permitiendo el mantenimiento de poblaciones con bajos índices de reproducción e incluso el restablecimiento de la especie en zonas donde haya sufrido un proceso de extinción local. Dichas zonas de exclusión de herbivoría deberían establecerse en las poblaciones Ep1, Ep2, Ep28 y Ep30, al ser las más importantes para la conectividad de la especie. Así como las poblaciones Ep03, Ep15, Ep 20 y Ep23, para garantizar la supervivencia en todas las zonas donde la especie está presente.

Otra medida eficaz para conservar la especia sería realizar una dispersión manual de semillas a comienzos de otoño, cuando los frutos están maduros. Esta medida sería especialmente importante en las poblaciones de la Sierra de Jabalcuz, Sierra Mágina y Sierra Arana, donde la limitación prioritaria es la limitación por dispersión (Capítulo 3). De llevarse a cabo esta dispersión manual, habría se depositar las semillas en zonas de matorral, para que se vean favorecidas por el efecto nodriza de los matorrales sobre los individuos de *E. popovii* (Capítulo 3).

También es muy importante completar estas investigaciones con la búsqueda de nuevas poblaciones en zonas intermedias donde nosotros no hemos encontrado. La búsqueda de estas nuevas poblaciones ha de centrase en la Sierra de Loja, zona norte de Sierra Arana, Sierra de Alta Coloma y Sierra de Parapanda. Esta búsqueda podría completar la presencia de la especie en la mayoría de zonas montañosas dentro de área de distribución. De no encontrar poblaciones de *E. popovii* en estas sierras podría plantearse la posibilidad de introducirlas, llevando semillas de otras zonas. Aunque para esto sería necesarias profundas investigaciones adicionales, incluyendo experimentos in-situ sobre la reintroducción de la especie.

Dada la situación extrema en la que se encuentra la población Ep11, se requiere una intervención especial en esta zona. Para garantizar la supervivencia de esta población será imprescindible la adición de semillas procedentes de otras poblaciones. Además debería de hacerse una búsqueda intensa de otras poblaciones en zonas colindantes y establecer un periodo de exclusión de herbivoría o al menos evitar el pastoreo hasta que la población se recupere.

Finalmente se hace necesario el realizar profundos estudios taxonómicos que analicen la relaciones filogenéticas con otras especies emparentadas como *E. baeticum* y *E. cazorlense*.

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CONCLUSIONES

1.- El éxito reproductivo de *Erysimum popovii* se correlacionó con la composición del conjunto de polinizadores y con la comunidad floral. La variación entre poblaciones generó un mosaico geográfico, con poblaciones con una alta tasa de reclutamiento y otras con un reclutamiento muy bajo, indicando un posible comportamiento metapoblacional.

2.- Los bombílidos parece ser el grupo de polinizadores más eficiente como vectores polínicos de *Erysimum popovii*. Altas tasas de visitas florales por parte de los bombílidos se correlacionó con un incremento en la emergencia de las plántulas y una disminución de la limitación por polen y limitación por semillas.

3.- La mayoría de las poblaciones sufren limitación por polen, lo que afecta de forma directa a la producción de semillas. Esta limitación mostró una variación geográfica a los largo del área de distribución de *Erysimum popovii.*

4.- De las 19 poblaciones en la que se estudió la limitación en el reclutamiento, 6 mostraron limitación por producción de semillas, 7 poblaciones limitación por dispersión de semillas y solo 6 limitación por disponibilidad de micrositios. Estas variaciones no mostraron un patrón geográfico a nivel regional.

5.- La abundancia de polinizadores de buena calidad fue superior en las poblaciones situadas dentro de espacios naturales protegidos. Sin embargo el daño por herbivoría también fue superior en estas poblaciones, lo que puede ser la causa de que *Erysimum popovii* no esté en mejor estado de conservación dentro de áreas protegidas.

6.- Muy pocas poblaciones de *Erysimum popovii* están por encima de la Población Mínima Viable. Por ello, la supervivencia de la especie a largo plazo solo es posible conservando poblaciones de gran tamaño y con una gran capacidad reproductiva, que actúen como fuente de propágulos favoreciendo un sistema de metapoblaciones.

7.- Para mejorar la conservación de la especie es recomendable el establecer zonas de exclusión de herbívora en las poblaciones más importantes. Además serían necesarias otras intervenciones específicas como la dispersión manual de semillas o incluso la translocación de semillas en regiones y poblaciones concretas.

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